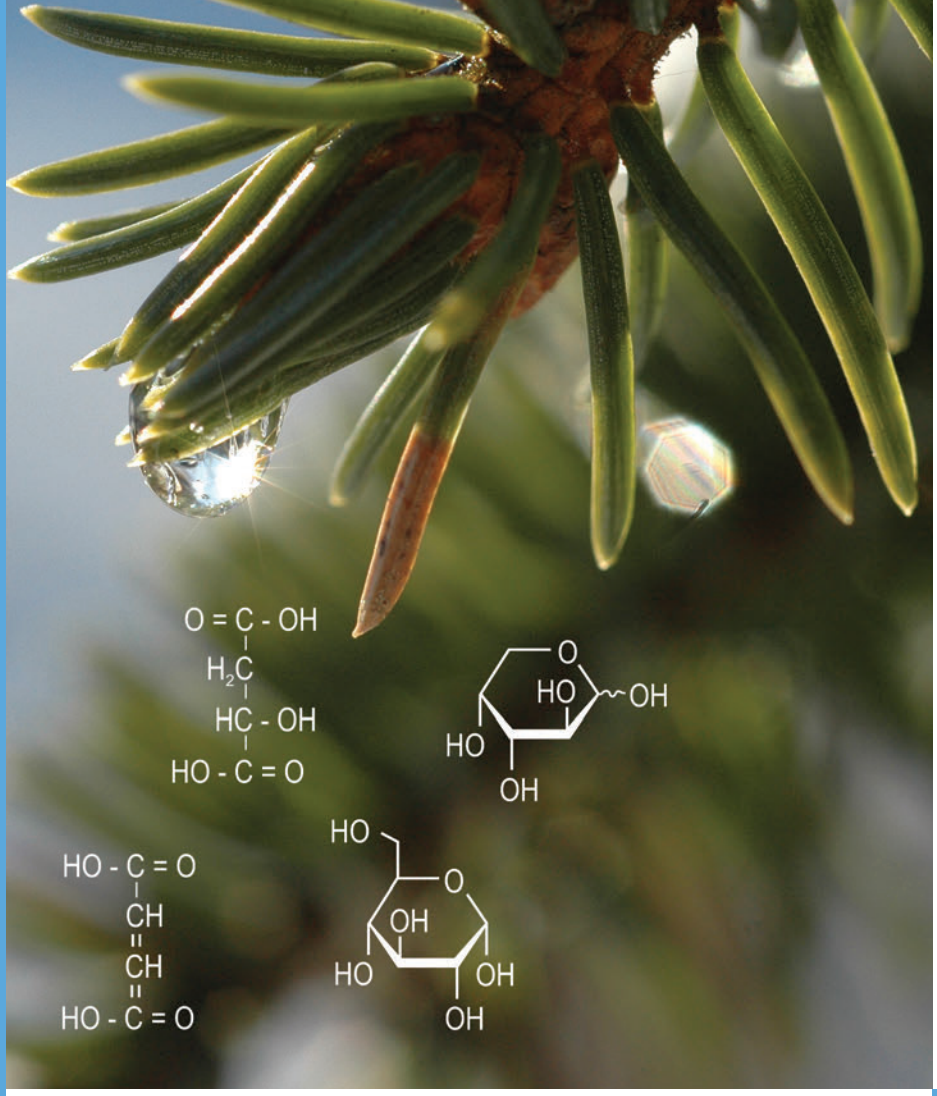

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Dissertation
21

Application of capillary electrophoretic methods for determining carbohydrates and aliphatic carboxylic acids formed during wood processing

Stella Rovio

VTT

VTT SCIENCE 21

Application of capillary electrophoretic methods for determining carbohydrates and aliphatic carboxylic acids formed during wood processing

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ACADEMIC DISSERTATION

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Application of capillary electrophoretic methods for determining carbohydrates and aliphatic carboxylic acids formed during wood processing

Kapillaarielektroforeettisten erotusmenetelmien soveltaminen puun prosessoinnissa syntyvien sokerien ja alifaattisten karboksyylihappojen määrityksessä. **Stella Rovio.** Espoo 2012. VTT Science 21. 112 p. + app. 70 p.

Abstract

Knowledge of the behaviour of cellulose, hemicelluloses, and lignin during wood and pulp processing is essential for understanding and controlling the processes. Determination of monosaccharide composition gives information about the structural polysaccharide composition of wood material and helps when determining the quality of fibrous products. In addition, monitoring of the acidic degradation products gives information of the extent of degradation of lignin and polysaccharides.

This work describes two capillary electrophoretic methods developed for the analysis of monosaccharides and for the determination of aliphatic carboxylic acids from alkaline oxidation solutions of lignin and wood.

Capillary electrophoresis (CE), in its many variants is an alternative separation technique to chromatographic methods. In capillary zone electrophoresis (CZE) the fused silica capillary is filled with an electrolyte solution. An applied voltage generates a field across the capillary. The movement of the ions under electric field is based on the charge and hydrodynamic radius of ions.

Carbohydrates contain hydroxyl groups that are ionised only in strongly alkaline conditions. After ionisation, the structures are suitable for electrophoretic analysis and identification through either indirect UV detection or electrochemical detection.

The current work presents a new capillary zone electrophoretic method, relying on in-capillary reaction and direct UV detection at the wavelength of 270 nm. The method has been used for the simultaneous separation of neutral carbohydrates, including mono- and disaccharides and sugar alcohols. The in-capillary reaction produces negatively charged and UV-absorbing compounds. The optimised method was applied to real samples. The methodology is fast since no other sample preparation, except dilution, is required.

A new method for aliphatic carboxylic acids in highly alkaline process liquids was developed. The goal was to develop a method for the simultaneous analysis of the dicarboxylic acids, hydroxy acids and volatile acids that are oxidation and degradation products of lignin and wood polysaccharides. The CZE method was applied to three process cases. First, the fate of lignin under alkaline oxidation conditions was monitored by determining the level of carboxylic acids from process solutions. In the second application, the degradation of spruce wood using alkaline and catalysed alkaline oxidation were compared by determining carboxylic acids from the process solutions. In addition, the effectiveness of membrane filtration and preparative liquid chromatography in the enrichment of hydroxy acids from black liquor was evaluated, by analysing the effluents with capillary electrophoresis.

Keywords Wood, capillary electrophoresis, carbohydrates, carboxylic acids, oxygen delignification, alkaline oxidation

Kapillaarielektroforeettisten erotusmenetelmien soveltaminen puun prosessoinnissa syntyvien sokerien ja alifaattisten karboksyylihappojen määrityksessä

Application of capillary electrophoretic methods for determining carbohydrates and aliphatic carboxylic acids formed during wood processing. **Stella Rovio**. Espoo 2012. VTT Science 21. 112 s. + liitt. 70 s.

Tiivistelmä

Käsitys selluloosan, hemiselluloosan ja ligniinin käyttäytymisestä puun käsittelyssä on oleellista, jotta prosesseja kyetään kontrolloimaan. Monosakkaridikoostumuksen määrittäminen antaa tietoa puun polysakkaridikoostumuksesta ja auttaa arvioimaan kuitutuotteiden laatua. Lisäksi happamien hajoamistuotteiden määrittäminen antaa tietoa ligniinin ja polysakkaridien hajoamisesta.

Tässä työssä kuvataan kahden kapillaarielektroforeettisen menetelmän kehitys monosakkaridien ja alifaattisten karboksyylihappojen määrittämiseksi ligniinin ja puun alkalihapetuksella käsitellyistä liuoksista.

Kapillaarielektroforeesi (CE) monine variaatioineen on vaihtoehtoinen erotustekniikka kromatografisille erotustekniikoille. Kapillaarivyöhyke-elektroforeesissa (CZE) silikakapillaari on täytetty elektrolyyttiliuoksella. Erotusjännite muodostaa sähkökentän kapillaarin päiden välille, jolloin ionien liikkuvuus sähkökentässä perustuu niiden varaukseen ja hydrodynaamiseen säteeseen.

Sokeriyhdisteiden hydroksyyliryhmät ionisoituvat vain hyvin emäksisissä olosuhteissa. Ionisoituminen helpottaa hiilihydraattien elektroforeettista analyysia, ja analyyttien monitorointi voidaan tehdä joko epäsuoraa UV-detektiota tai sähkökemiallista monitorointia käyttäen.

Tutkimuksessa esitellään uusi kapillaarielektroforeettinen erotusmenetelmä sokerianalytiikkaan. Menetelmässä käytetään vahvasti emäksistä elektrolyyttiliuosta ja sokeriyhdisteet monitoroidaan suoraa UV-detektointia käyttäen 270 nm aallonpituudella. Käytetyt erotusolosuhteet ovat sellaiset, että mono- ja disakkaridit sekä sokerialkoholit varautuvat negatiivisesti ja niistä muodostuu UV-absorboivia yhdisteitä. Optimoitua menetelmää testattiin todellisilla näytteillä, joista mitattiin valikoitujen hiilihydraattien pitoisuudet. Tutkielmassa esitetty erotusmenetelmä on nopea, koska ainoa esikäsittelyvaihe ennen analyysia on laimentaminen.

Alifaattisten karboksyylihappojen määrittämiseksi emäksisistä prosessiliuoksista kehitettiin uusi kapillaarielektroforeettinen erotusmenetelmä. Tavoitteena oli luoda menetelmä, jolla kyetään erottamaan yhdellä ajolla dikarboksyylihapot, hydroksihapot sekä haihtuvat hapot, joita syntyy ligniinin ja puun polysakkaridien hajoamis- ja hapettumisreaktioissa. Kehitettyä CZE-menetelmää sovellettiin kolmessa prosessissa. Ensimmäinen applikaatio käsitti karboksyylihappojen määrittämisen prosessiliuoksesta, kun ligniiniä hapetettiin alkalisessa liuoksessa. Toisessa sovelluksessa verrattiin katalysoitua alkalista hapetusta ei-katalysoituun alkaliseen hapetukseen määrittämällä alifaattisia karboksyylihappoja prosessiliuoksista.

Karboksyylihappomäärityksiä käytettiin myös fraktioanalyyseissä tutkittaessa kalvosuodatuksen ja preparatiivisen nestekromatografian käyttökelpoisuutta karboksyylihappojen rikastamisessa mustalipeänäytteistä.

Avainsanat Wood, capillary electrophoresis, carbohydrates, carboxylic acids, oxygen delignification, alkaline oxidation

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Academic dissertation

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Custos	Professor Heli Sirén

List of papers

This thesis is based on the following original papers which are referred to in the text as I–VI.The papers are reproduced with kind permission from the publishers. Some additional, unpublished results are included.

I Rovio, S., Yli-Kauhaluoma, J., Sirén, H. Determination of neutral carbohydrates by CZE with direct UV detection. *Electrophoresis* 28 (2007) 3129–3135.

II Rovio, S., Simolin, H., Koljonen, K., Sirén, H. Determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis. *Journal of Chromatography A* 1185 (2008) 139–144.

III Rovio, S., Kalliola, A., Sirén, H., Tamminen, T. Determination of the carboxylic acids in acidic and basic process samples by capillary zone electrophoresis. *Journal of Chromatography A* 1217 (2010) 1407–1413.

IV Rovio, S., Kuitunen, S., Ohra-aho, T., Alakurtti, S., Kalliola, A., Tamminen, T. Lignin oxidation mechanisms under oxygen delignification conditions. Part 2: Advanced methods for the detailed characterization of lignin oxidation mechanisms. *Holzforschung* 65 (2011) 575–585.

V Rovio, S., Kallioinen, A., Tamminen, T., Hakola, M., Leskelä, M., Siika-aho, M. Catalysed alkaline oxidation as a wood fractionation technique. *BioResources* 7 (2012) 756–776.

VI Niemi, H., Lahti, J., Hatakka, H., Kärki, S., Rovio, S., Kallioinen, M., Mänttäri, M., Louhi-Kultanen, M. Fractionation of organic and inorganic compounds from black liquor by combining membrane separation and crystallization. *Chemical Engineering & Technology* 34 (2011) 593–598.

The author's contribution in the original papers

I The author invented the original idea, carried out the method development, and wrote the Paper together with the co-authors.

II The author carried out the capillary electrophoresis experiments and wrote the paper together with the co-authors.

III The author carried out the capillary electrophoresis method development and wrote the paper together with the co-authors.

IV The author carried out the capillary electrophoresis experiments and part of the ^{31}P NMR measurements and wrote the paper together with the co-authors.

V The author carried out the capillary electrophoresis experiments of the dissolved acidic compounds and wrote the paper together with the co-authors.

VI The author carried out the capillary electrophoresis experiments of the fractionated acidic compounds and wrote the paper together with the co-authors.

Other related publications

Rovio, S., Sirén, K., Sirén, H. Application of capillary electrophoresis to determine metal cations, anions, organic acids, and carbohydrates in some Pinot Noir red wines. *Food Chemistry* 124 (2011) 1194–1200.

Sirén, H., Sirén, K., Sharma, S., Kaijanen, L., Ruokonen, J., Bricka, M., Rovio, S., Differences in Pinot Noir Red Wines Produced by Different Methods: Chromatographic, Spectroscopic, and Electro Aided Study. In: Peeters, A.S. (ed.) Wine: Types, Production and Health, Nova Science Publishers, NY, USA, 2011.

Contents

Appendix IV is not included in the PDF version.
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List of abbreviations

2-AP	2-Aminopyridine
ABEE	4-aminobenzoic acid ethyl ester
AlkOx	Alkaline oxidation
ANTS	8-Aminonaphthalene-1,3,6-trisulfonic acid
APTS	8-Aminopyrene-1,3,6-trisulfonate
ARA	Arabinose
BGE	Background electrolyte
CatOx	Catalysed alkaline oxidation
CCD	Contactless conductivity detection
CE	Capillary electrophoresis
CEL	Cellobiose
CTAB	Cetyltrimethylammonium bromide
CZE	Capillary zone electrophoresis
2,5-DHPA	2,5-Dihydroxypentanoic acid
EOF	Electroosmotic flow
FID	Flame ionisation detector
FUC	Fucose
GAL	Galactose
GC	Gas chromatography
GISA	Glucoisosaccharinic acid
GLU	Glucose
i.d.	Internal diameter

IUPAC	International Union of Pure and Applied Chemistry
HPAEC	High-performance anion-exchange chromatography
HPLC	High-performance liquid chromatography
LEC	Ligand-exchange chromatography
LIF	Laser induced fluorescence
MAN	Mannose
MS	Mass spectrometry
NAA	1-Naphthylacetic acid
nm	Nanometer (10^{-9} m)
NMR	Nuclear magnetic resonance
o.d.	Outer diameter
PAD	Pulsed amperometric detector
^{31}P NMR	Phosphorous nuclear magnetic resonance spectroscopy
2,3-PyDC	2,3-Pyrazinedicarboxylic acid
2,3-PDC	2,3-Pyridinedicarboxylic acid
2,6-PDC	2,6-Pyridinedicarboxylic acid
PS-DVB	poly(styrenedivinylbenzene)
RI	Refractive index
RIB	Ribose
RHA	Rhamnose
TEA	Triethanol amine
TIC	Total ion current
TMP	Thermo mechanical pulp
UV	Ultraviolet
v/v	volume / volume ratio
XISA	Xyloisosaccharinic acid
XYL	Xylose

List of symbols

α	Degree of dissociation
C(1) – C(6)	Carbon number in carbohydrate molecule, n=1-6
D-	*Dextro-*
ΔT	Temperature difference
E	Electric field strength (V/cm)
ε	Dielectric constant
I	Ionic strength
κ	Conductivity
L-	*Levo-*
L_{det}	Capillary length to detection point
L_{tot}	Total capillary length
μ_{act}	Actual mobility of the fully charged acid or base
μ_{app}	Apparent mobility
μ_{eff}	Effective mobility
μ_{eo}	Electroosmotic mobility
η	Stern layer
pKa	Dissociation constant
r	Capillary radius
υ	Migration velocity
ζ	Zeta potential
V	Voltage

1. Introduction

Trees are perennial plants, which are classified into two broad classes: softwoods (“evergreen” trees) and hardwoods (“deciduous” trees). The principal components of wood are the polysaccharides, cellulose, (a homopolymer of glucose), and hemicelluloses, which are constructed mainly from glucose, mannose, arabinose, rhamnose, and xylose. Lignins are the third major constituent of wood, a class of polymers with considerable structural diversity.

Extractives, proteins, and inorganic compounds are also found, but only in minor amounts.

In chemical pulping processes, lignin is removed from wood and fibres are separated from each other. Less than ten per cent of the original lignin is retained in fibres after Kraft pulping. This residual lignin is removed using an oxygen-alkali delignification process. By the action of oxygen, the lignin polymer is degraded so that it becomes more hydrophilic. Thereafter, it can be removed by solubilisation. As a result of the degradation, a substantial amount of aliphatic carboxylic acids can be found in solution.

Oxygen-alkali treatment is also an important pre-treatment method for the production of cellulosic ethanol from lignocellulosic materials. Oxygen-alkali pre-treatment, involves the use of bases, such as sodium-, potassium-, or calcium hydroxides or calcium carbonate. The hydrolysis of lignocellulosic material can be enhanced using a copper-based catalyst. By the action of alkali and oxygen, lignin and hemicelluloses are degraded and produce a large selection of dissolved aliphatic carboxylic acids in solution.

Capillary zone electrophoresis (CZE) is one of the sub techniques of capillary electrophoresis (CE). Because of its simplicity, CZE is a suitable technique for separating charged and water-soluble compounds. The separation of compounds under an electric field is based on differences in their charge-to-size ratios.

During the past twenty years, several CE methods have been introduced to quantify neutral carbohydrates. Two main separation strategies have been applied. The first uses strongly alkaline conditions, with the detection based on either indirect UV or on electrochemical detection. The other separation principle is based on the derivatization of carbohydrates with an ionisable and UV absorbing tag, which leads to use of only moderately alkaline analysis conditions. The current study presents an alternative CE method to the above-mentioned, estab-

lished, capillary electrophoretic methods for the analysis of mono-, di-, and oligosaccharides and sugar alcohols. The method relies on in-situ formation of a UV absorbing derivative in a medium of extreme alkalinity. Identification is made then by direct UV detection. The applicability of the method is demonstrated in the analysis of selected carbohydrates in the quantification of neutral monosaccharides in cellulosic fibres of different origin liberated by acid hydrolysis.

For the determination of various aliphatic carboxylic acids in wood processed solutions, a new CE method is developed. The method is able to separate volatile acids, dicarboxylic acids and hydroxy acids simultaneously together with common inorganic anions found in various wood process solutions.

2. Review of the literature

2.1 Monosaccharides

Cell wall polysaccharides are composed of pentose and hexose monosaccharides. Carbohydrates have important roles in plants, as they serve as building blocks, energy stores and they participate in many cell functions in biological systems.

Carbohydrates are polyhydroxylated aldehyde or ketone compounds. The simplest carbohydrates are monosaccharides, which contain three to seven carbon atoms. According to carbon number, they are called trioses, tetroses, pentoses, hexoses, or heptoses. Monosaccharides are further divided into two groups: aldoses and ketoses. Aldoses have an aldehyde group at C(1) while ketoses contain a keto group at C(2) (Figure 1). The most common aldose is glucose, an aldohexose, and fructose is an example of a ketohexose. D-carbohydrates, which differ from each other in the configuration at a single symmetric centre, are epimers [1, 2].

Sugar alcohols, also called polyols, are compounds obtained by reduction of the corresponding aldoses and ketoses. Common sugar alcohols are mannitol (from mannose), glucitol, also known as sorbitol (from glucose), and xylitol (from xylose) [3].

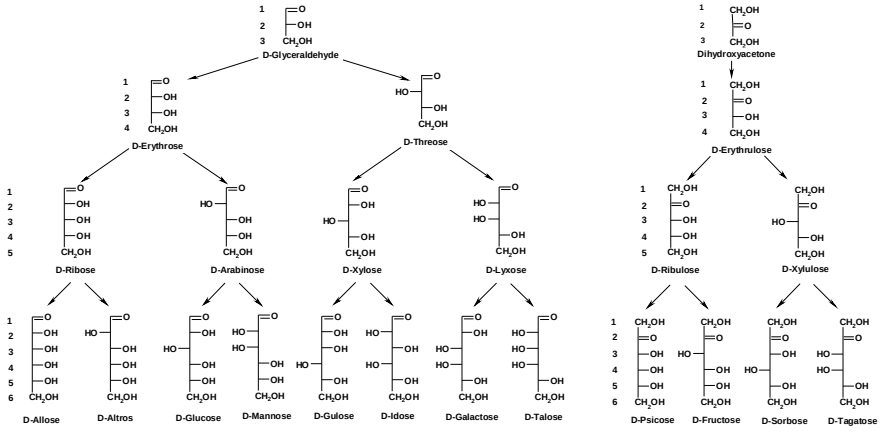

Figure 1. Acyclic forms of D-aldoses (left tree) and D-ketoses (right) containing 3–6 carbon atoms in the carbon chain. Modified from ref. [1].

The term “neutral monosaccharide” or “neutral sugar” is generally associated with carbohydrates that do not carry any charged group on the molecule. The dissociation constants (pKa values) of carbohydrates are around 12–14 and with increasing hydroxide concentration several of the hydroxyl groups become ionised, the C(2) being the most acidic.

Table 1 lists the pKa values of some carbohydrates. The anomeric centres C(1) of aldoses and C(2) of ketoses are the most reactive sites within monosaccharide molecules [1]. In aqueous alkaline solutions, monosaccharides undergo both reversible and irreversible transformations (Figure 2). Reversible reactions include ionisation, mutarotation, and enolisation [4, 5]. Usually, reducing carbohydrates (e.g. sugars having an aldehyde group which allows the carbohydrate to act as a reducing agent [6], such as glucose, galactose, and mannose) are the most-easily ionised. The higher acidity of reducing carbohydrates is caused by the higher lability of the hydrogen atom of the anomeric hydroxyl group at C(1), a condition that stems from an electron-withdrawing polar effect exerted upon this group by the ring oxygen [7].

In addition to the ionisation reaction and mutarotation, alkali catalyses the loss of hydrogen from an anomeric hydroxyl group, which leads to mutarotation of hemiacetals and hemiketals. At millimolar concentrations of hydroxide anion, the mutarotation of carbohydrates is very rapid [1, 4, 8].

Table 1. Dissociation constants of selected carbohydrates.

Compound	**pK_a** ***(in water at 25°C)***	**Reference**	
D-(-)-Arabinose	12.43	9	
Fructose	12.03	9	
D-(+)-Galactose	12.35	9	
D-(+)-Glucose	12.35, 12.28	9, 10	
Mannitol	13.50[a]	7	[a] measured in water at 18 °C
D-(+)-Mannose	12.08	9	
D-(-)-Ribose	12.21	9	
Sucrose	12.51	9	
Xylitol	13.8[b]	11	[b] measured in water at 22.5 °C
D-(+)-Xylose	12.29, 12.15	9, 10	

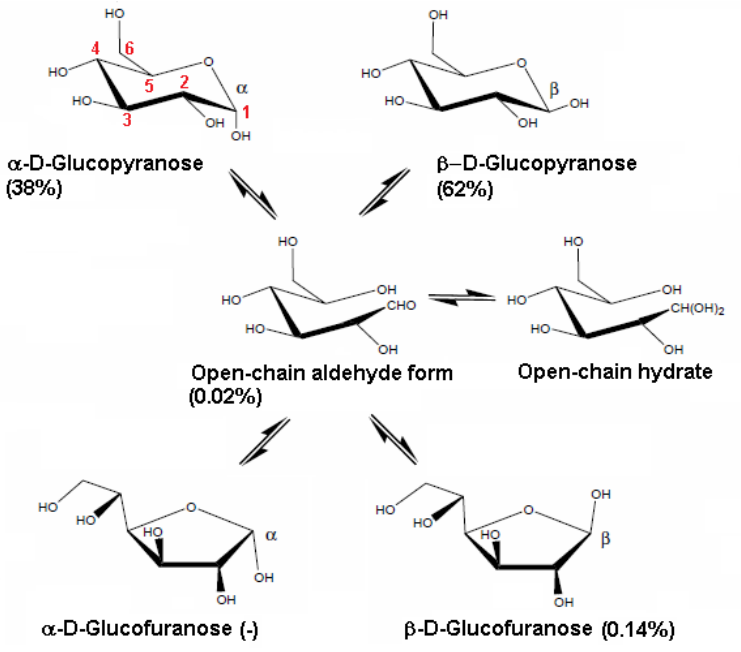

Figure 2. Mutarotation of D-glucose to the different pyranose and furanose forms in aqueous solution. Percentages present the amount (in %) of the different structures at the equilibrium state [1].

2.2 Chemical composition of wood

The principal structural components of wood are cellulose, hemicelluloses and lignin, together with a smaller amount of pectic substances and extractives [12]. Depending on tree species, the percentage ratios of these components differ to some extent (Table 2). Extractives, proteins, and inorganic compounds are also found, but only in minor amounts.

Table 2. Typical structural components of softwood and hardwood, presented as percentage of dry wood [adapted from ref. 13].

Type of Wood type	Cellulose	Hemicelluloses		Other polysaccharides	Lignin
		Glucomannan	Xylan		
Softwood	33–42	14–20 [a)]	5–11 [b)]	3–9	27–32
Hardwood	38–51	1–4 [c)]	14–30 [d)]	2–4	21–31

a) Galactoglucomannan: galactose-rich fraction and galactose-poor fraction (glucomannan)
b) Arabinoglucuronoxylan
c) Glucomannan
d) Glucuronoxylan

2.2.1 Cellulose

Cellulose is the main polysaccharide in wood. It is the major constituent in plant cell walls giving the structural strength [13]. Cellulose is a linear homopolymer of β-1,4-D-glucose units. Each glucose unit is related to the next one by a rotation of 180° and the chain structure is further fortified with the hydrogen bonds between ring oxygen and OH unit attached to C(3) in the next unit (Figure 3) [1]. Long cellulose molecules are aggregated to form fibrils, which bind to each other by hydrogen bonds and van der Waals forces. A highly regular hydrogen-bonded network between the layers of cellulose and its crystalline nature mean that cellulose is insoluble in water. However, it can be hydrolysed using 70% sulphuric acid [1].

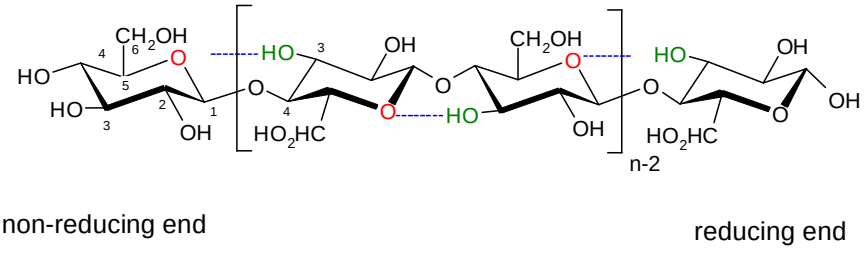

Figure 3. Structure of cellulose. The structure is stabilised by the hydrogen bonds (dotted line) between adjacent glucose units [1].

2.2.2 Hemicelluloses

2.2.2.1 Hemicelluloses in softwoods

Hemicelluloses are heteroglycans exhibiting an amorphous, branched structure consisting of the monosaccharides glucose, mannose, and galactose, which are hexoses, and pentoses like xylose and arabinose. The monosaccharides are linked to each other, for example, with β-1,4-linkages. In addition, acidic residues such as 4-O-methylglucuronic acid and galacturonic acid can be found in hemicellulose structures. Galactoglucomannan and glucomannan are principal hemicelluloses in softwood. These two glucomannans are differentiated in respect of their galactose content. In addition, some of the mannose and glucose units are substituted with acetyl groups, typically, on average one group per 3–4 hexose units [12].

The third major hemicellulose in softwood is arabinoglucuronoxylan, or xylan for short [12]. Figure 4 depicts a schematic diagram of the major hemicellulose of softwood. Arabinogalactan, xyloglucan, other glucans, and pectins are found in smaller quantities in softwood. Hemicelluloses are present between the cellulose fibrils in the cell wall [14]. Unlike cellulose, hemicelluloses are easily hydrolysable polymers due their lower aggregability [1].

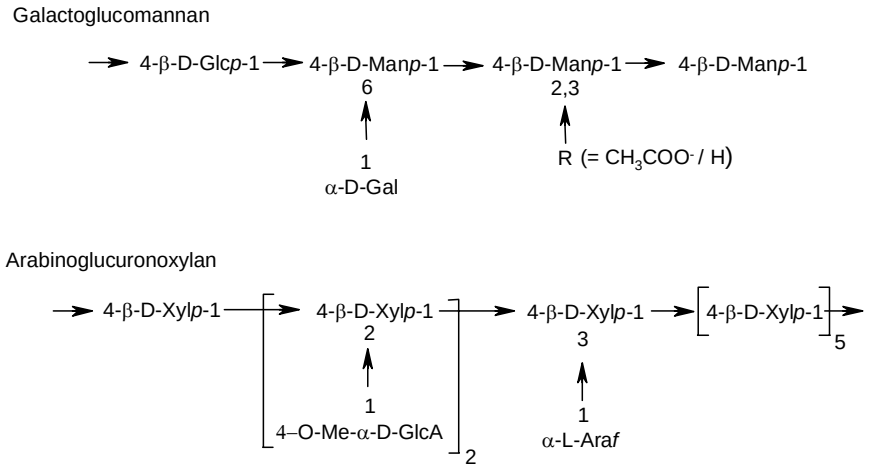

Figure 4. Structures of hemicelluloses galactoglucomannan and arabinoglucuronoxylan in softwood. Carbohydrate units: Glcp: β-D-glucopyranose, Manp: β-D-mannopyranose, Gal: β-D-galactopyranose, Xylp: β-D-xylopyranose, 4-O-Me-α-D-GlcA: 4-O-methyl-α-D-glucuronic acid, Araf: α-L-arabinofuranose [12, 13].

2.2.2.2 Hemicelluloses in hardwood

Hemicelluloses of hardwood differ from those of softwood in respect of their nature and the percentage proportions in which they are found in woods. Hardwood's hemicelluloses typically contain more xylan and acetyl groups than softwood hemicelluloses, which, in turn, have higher proportion of mannose and galatose [12].

The major hemicellulose in hardwood is *O*-acetyl-4-*O*-methylglucurono-β-D-xylan, glucuronoxylan for short. The backbone consists of (1 → 4) linked β-D-xylopyranose units. On average, seven out of 10 xylose units contain an *O*-acetyl group at C(2) or at C(3). In addition, a (1→ 2) linked 4-*O*-methyl-α-D-glucuronic acid residue is attached to every tenth xylose unit [12, 13]. In addition, the reducing end of glucuronoxylan contains a dimeric segment consisting of an α-L-rhamnose linked to an α-D-galacturonic acid residue (Figure 5). Another hemicellulose found in hardwood is glucomannan. It has a similar linear backbone to softwood's galactoglucomannan (Figure 5), but with a higher glucose-mannose ratio, which typically varies between 1:2 and 1:1 [13]. Hardwood glucomannan is *O*-acetylated at the C-2 and C-3 positions of certain of their mannose residues [15].

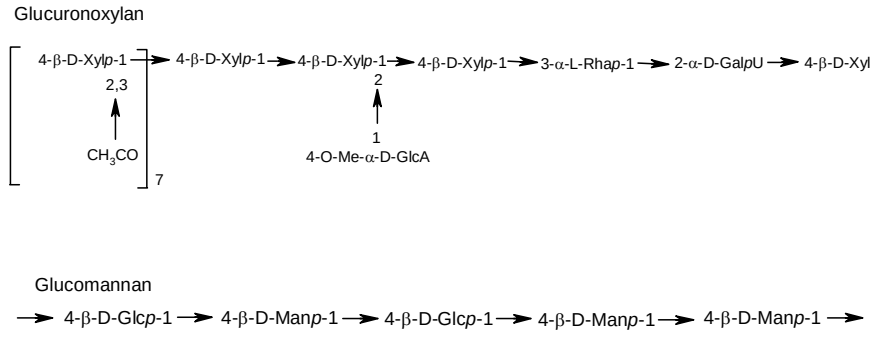

Figure 5. Structures of hardwood hemicelluloses. Carbohydrate units as in Figure 4 [12, 13].

2.2.3 Lignin

In addition to the polymeric carbohydrates, lignin is the third major component of lignocellulosic biomass. Its main function is to bind cellulose and hemicellulose fibres and give strength to the plant cell wall, hydrophobicity and resistance against microbial decay. Lignin provides hydrophobic regions for the formation of transport channels for water and extracellular fluids [16, 17].

Lignin is a three-dimensional amorphous heteropolymer consisting of methoxylated phenyl propane structures with varying degrees of substitution on the aromatic ring. Dehydrogenation products of three aromatic monomers, namely p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol dominate its composition [18]. These monolignols are precursors for corresponding phenyl propane: guaiacyl (4-hydroxy-3-methoxyphenyl, G) syringyl (4-hydroxy-3,5-dimethoxyphenyl, S) and 4-hydroxyphenyl (H) units in lignin. Softwood lignins consist almost exclusively of G units, whereas hardwood lignins consist of various proportions of G and S units. Annual plants are built up from H, G, and S units [16, 19]. The phenyl propane units are linked to each other via multiple linkages, such as phenyl coumaran (β-5), resinol (β-β), arylglycerol-β-aryl ether (β-O-4), and biphenyl (5-5'). The distribution of these linkages varies between softwood and hardwood lignin. Among these linkages, the β-O-4 linkage is dominant both in softwood and hardwood lignin [20, 21]. The structure of a lignin macromolecule is characterised as irregular and disordered. A schematic picture of softwood and hardwood lignin structures, their monolignol building blocks and common bonds are presented in Figure 6.

Lignin is linked with cellulose and hemicellulose through chemical bonds. Carbohydrates are connected to lignin through ether- and ester-linkages, such as benzyl-ester, benzyl-ether, and phenyl-glycoside [16, 22]. Ether linkage is considered one of the main stable crosslinks between lignin and plant polysaccharides.

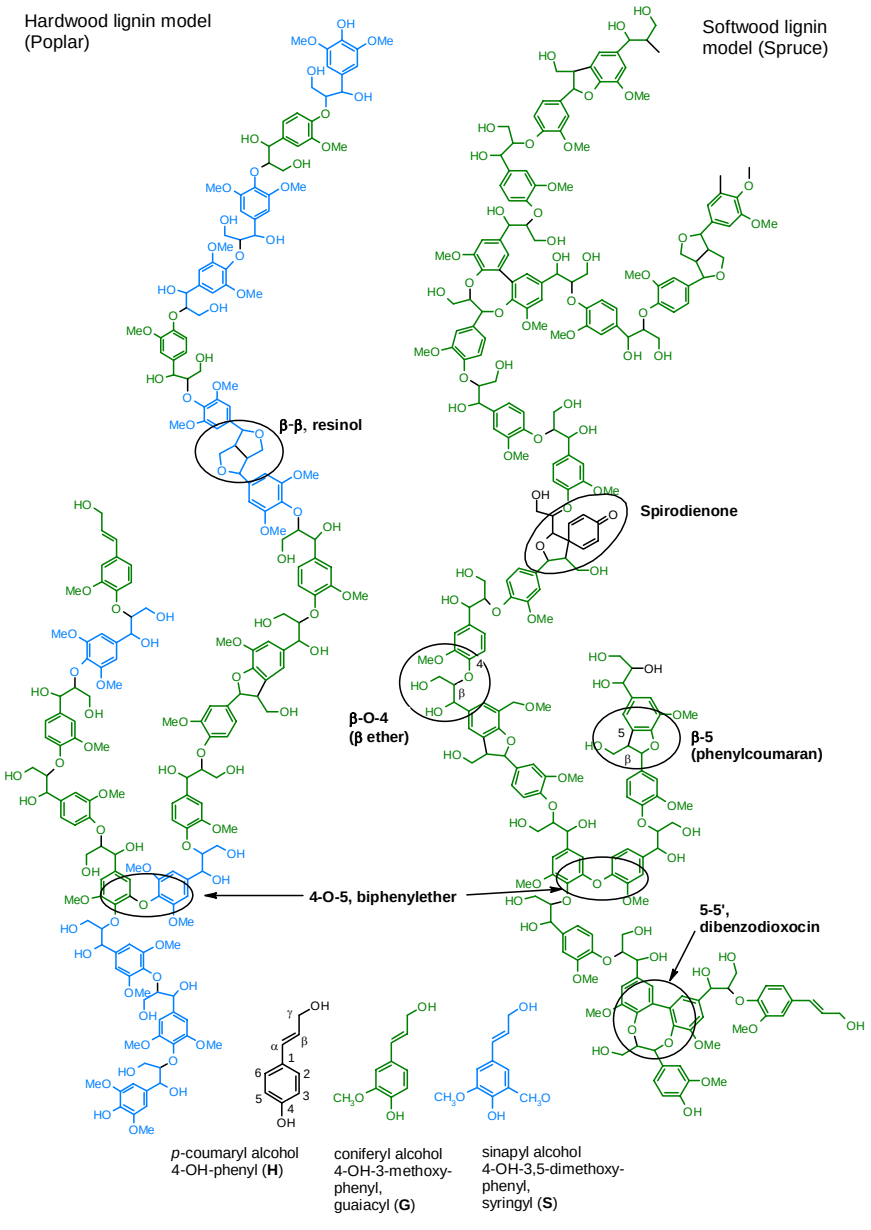

Figure 6. The three monolignols, the lignin polymer models of hardwood (poplar) lignin and softwood lignin (spruce), and the common linkages [23].

3. Kraft pulping process

The sulphate cooking, Kraft process is the dominant alkaline pulping process for wood. The process is used for both hardwood and softwood digestion. Principal cooking chemicals are sodium hydroxide and sodium sulphide, Na_2S. Typically, the Kraft cooking is carried out at temperatures of 155–175 °C and pressures of 7–11 bar [12, 13]. The digestion time varies between 2–5 hours. The principal digester can work either as a batch or continuous system. The yield of high quality chemical pulp is 45–55% of wood input, by weight [24].

The reactions that occur during Kraft pulping are complex. However, it is determined that hydrogen sulphide ion (HS^-) primarily degrades lignin whereas hydroxy anions (OH^-) are responsible for the reactions of hemicelluloses, i.e. cleavage of acetyl groups and peeling reactions. During Kraft pulping, about half of the wood substance degrades and dissolves. A substantial amount of hemicellulose is converted to aliphatic hydroxy acids and only small portion of dissolved polysaccharides can be found in the spent liquor. According to Alén, softwood Kraft black liquor dry matter contains 2% of cellulose and hemicellulose derived carbohydrate fragments whereas the portion of aliphatic hydroxy acids is around 16% [24].

The spent cooking liquor, black liquor, consists of lignin, carbohydrate degradation products, resin and fatty acids [13]. After the recovery of the tall oil fraction and turpentine from softwood black liquor, the remained Kraft black liquor is typically burned and the energy released is used for the process. In addition to above mentioned by-products, small amounts of kraft lignin are also isolated from black liquors for chemical applications [25]. However, it is also a potential source of hydroxy acids for non-fuel purposes. The black liquor of softwood contains ca. 29 weight-% aliphatic carboxylic acids of dry solids [24]. Therefore, the recovery of acids seems attractive. Table 3 shows a typical composition of pine and birch Kraft black liquors.

Table 3. Composition of pine and birch Kraft black liquors (percentage of dry matter). The composition of carboxylic acid fraction is further divided in volatile acids, monocarcarboxylic acids, and dicarboxylic acids. The most abundant carboxylic acids are highlighted in bold [24].

Component		**Pine**	**Birch**
Inorganics		33	33
Organics		67	67
	Lignin	31	25
	Extractives	4	3
	Polysaccharides	2	7
	Other organics	1	1
	Aliphatic carboxylic acids	29	29
	Volatile carboxylic acids		
	Formic	6	4
	Acetic	4	8
	Monocarboxylic acids		
	Glycolic	2	2
	Lactic	3	3
	2-Hydroxybutanoic	1	5
	2,5-Dihydroxypentanoic (=3,4-dideoxypentonic acid)	2	1
	Xyloisosaccharinic	1	3
	Glucoisosaccharinic	6	3
	3-Hydroxypropanoic	(*	(*
	Glyceric		
	4-Hydroxybutanoic		
	2-Deoxytetronic		
	4-Deoxytetronic		
	2,4,5-Trihydroxypentanoic		
	2-Hydroxypentenoic		
	Anhydroisosaccharinic		
	3,6-Dideoxyhexonic		
	3-Deoxyhexonic		
	Dicarboxylic acids		
	Oxalic		
	Succinic		
	Methylsuccinic		
	Malic		
	2-Hydroxyglutaric		
	2-Hydroxyadipic		
	2,5-Dihydroxyadipic		
	Glucoisosaccharinaric		

(* percentage portion of these acids is below 1%.

3.1 Degradation reactions of lignin in Kraft cooking

During the Kraft cooking, the dissolution of lignin proceeds in three phases. In the initial phase, also called the extraction phase, the lignin content is decreased only by 15–25% from the initial amount. The second phase is called the bulk delignification phase, in which about 90% of lignin is removed. During this phase, the reaction temperature is above 140 °C and the delignification is controlled by chemical reactions. The third phase is called residual delignification and the delignification rate here is much lower than in the preceding phase [24].

The delignification reactions can be divided into two categories: degradation reactions that proceed through the cleavage of inter unit linkages and condensation reactions [26]. In the degradation reactions, the hydroxide and hydrosulphide anions react with the lignin, causing the polymer to fragment into smaller water/alkali-soluble fragments while the condensation reactions are less desirable, since they lead to formation of alkali-stable linkages [27].

3.2 Degradation reactions of polysaccharides in Kraft cooking

Besides delignification, Kraft cooking leads to degradation of the polysaccharides. The alkaline degradation processes mainly affect hemicelluloses, while cellulose is more stable against degradation – due the crystalline nature and higher degree of polymerization. Approximately 30% of wood polysaccharides are converted into carboxylic acids, which are the main degradation products of polysaccharides [24, 28].

The formation of acids starts at very beginning of the Kraft cook, when the acetyl groups of softwood's galactoglucomannan and hardwood's xylan are hydrolysed [13]. The degradation reactions of the wood polysaccharides under Kraft pulping conditions can be categorised by the following subreactions:

I) end-wise degradation, i.e. peeling reactions of the reducing end (primary peeling), i.e. stepwise elimination of the monosaccharide unit,

II) stopping reaction that competes with peeling reactions and

III) random cleavage of glycosidic bonds of the polysaccharide chain by alkaline hydrolysis. The random cleavage of polysaccharide chain produces new reducing ends, which are further exposed to peeling (secondary peeling) [24, 28].

In the peeling reaction, the reducing end group is removed. The initial step is the keto-enol tautomerization of a reducing end group. The steps that follow include enediol deprotonation, anion isomerisation and β-alkoxy carbonyl elimination. The released intermediate is further exposed again to a reaction cascade that includes keto-enol tautomerism and benzilic acid rearrangements before the formation of hydroxy acids, such as glucoisosaccharinic or xyloisosaccharinic acid [28, 29]. The β-alkoxy carbonyl cleavage as well as the random cleavage of the carbohydrate chain by alkaline hydrolysis produces new reducing ends, which are again vulnerable to the peeling reaction [28].

The degradation of the polysaccharide chain is interrupted when the reducing end group forms a metasaccharinic acid through β-hydroxycarbonyl elimination (β-elimination) and benzilic acid rearrangement. Other routes proceed via similar reactions through glycol aldehyde intermediates to methylglyceric end groups [28].

Figure **7** summarises different subreactions in the reaction cascade leading to the degradation oligosaccharide chain and finally to carboxylic acids, while Figure 8 shows a simplified scheme of the main reactions of the peeling and stopping reaction paths of polysaccharides during Kraft pulping.

Table 4 summarises different alkaline degradation reactions of wood material, polysaccharides and monosaccharides found in wood polysaccharides. The reaction products are a complex mixture of volatile monocarboxylic acids, hydroxy acids and dicarboxylic acids. As can be seen in Table 4, the composition of the alkaline degradation products are influenced by several reaction parameters, such as the hydroxyl ion concentration, temperature and nature of the alkali. However, the main degradation products belong to the group of hydroxy acids. In addition, formic and acetic acids are among the most abundant degradation products. The black liquor also contains several dicarboxylic acids, although in rather small amounts. Among them, the most abundance acids are oxalic, succinic, malic, methylsuccinic and 2,5-dihydroxyadipic acids. The dicarboxylic acids are identified as degradation products of pectic substances, cellulose, and cellobiose [30, 31]. The presence of divalent cations, such as calcium and magnesium, accelerates the degradation of monosaccharides and influences the final product composition [32, 33, 34].

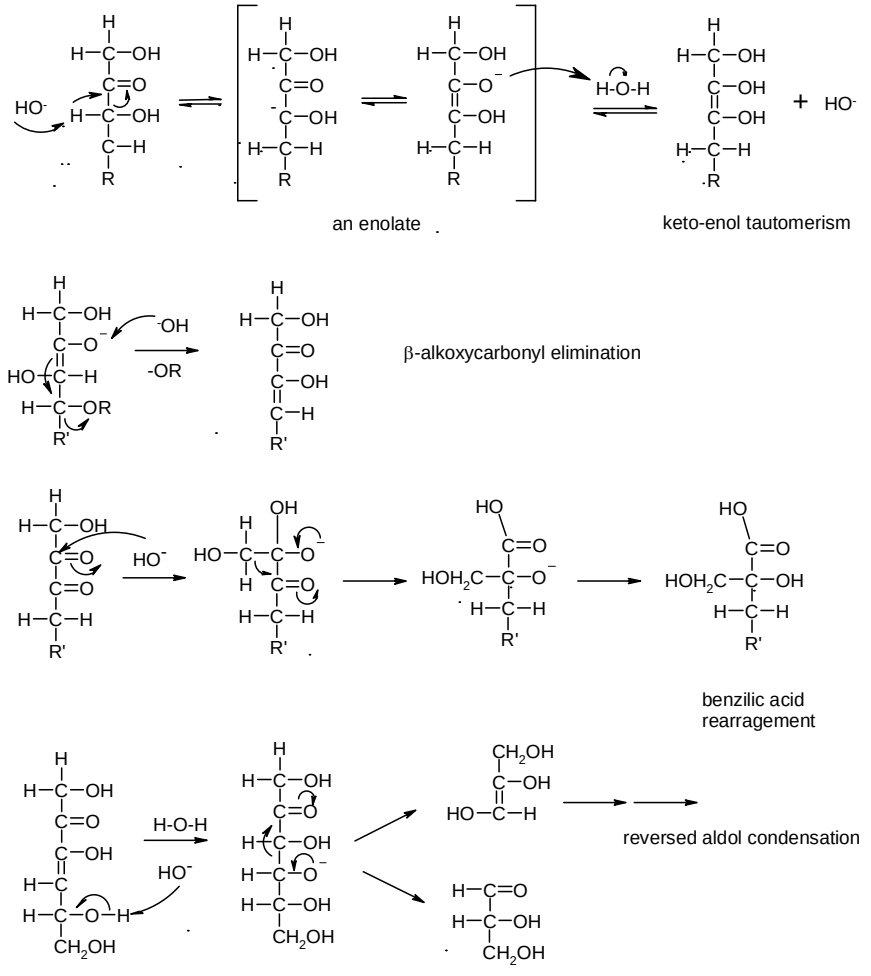

Figure 7. Rearrangements of carbohydrates in an aqueous alkaline medium [35].

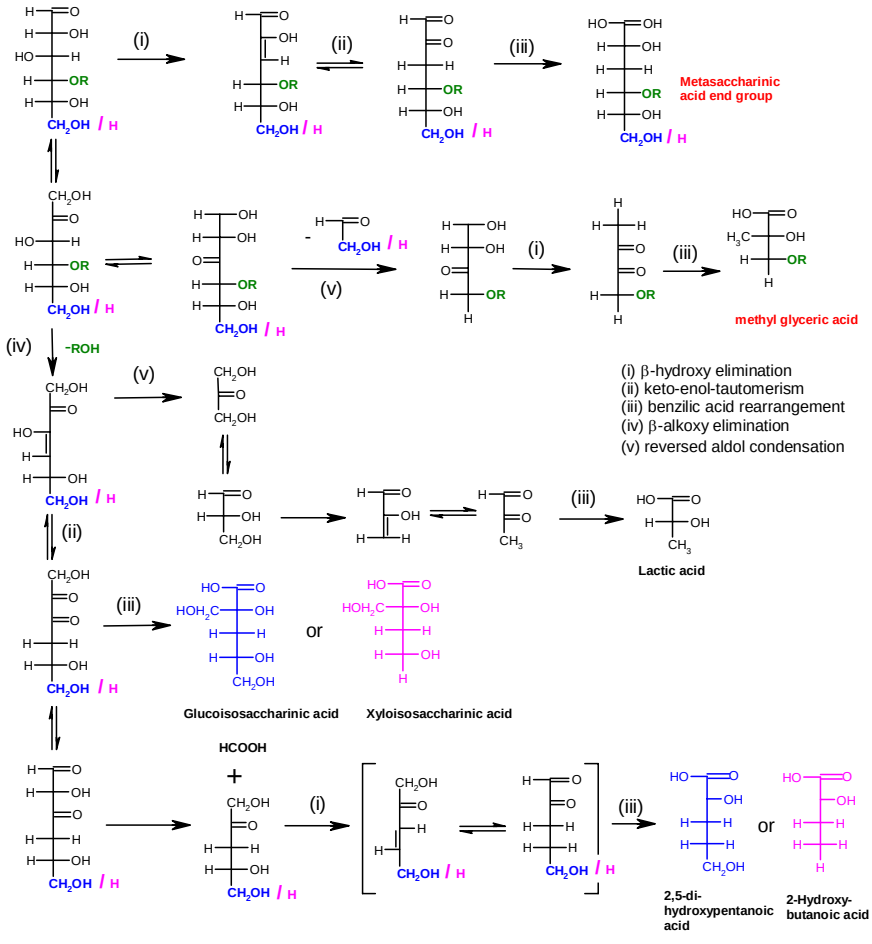

Figure 8. A schematic diagram of the peeling and stopping reaction paths of polysaccharides during Kraft pulping. The end groups of stopping reactions are displayed with a red colour. R = polysaccharide chain, blue end group at C(6): cellulose and glucomannan, magenta end group at C(6): xylan [28, 29].

Table 4. Degradation of softwood and hardwood, wood polysaccharides and monosaccharides related to wood polysaccharides under various alkaline conditions. The most abundant aliphatic carboxylic acids are grouped as volatile acids, hydroxy acids, and dicarboxylic acids.

Acids: Volatile acids: C1 Formic; C2a Acetic. Hydroxy acids: C2b Glycolic; C3a Glyceric; C3b Lactic; C3c 2,3-Dihydroxypropenoic; C4a 2-Hydroxybutanoic; C4b 2-Hydroxyisobutanoic; C4c 2,4-Dihydroxybutanoic; C4d 3,4-Dihydroxybutanoic; C4e2-C-Methylglyceric; C4f Erythronic; C4g Threonic; C4h Deoxy-tetronic; C5a 2,5-Dihydroxypentanoic; C5b 2-Hydroxypentanoic; C5c 3-Hydroxypentenoic; C5d 2,4,5-Trihydroxypentanoic; C5e 3-Deoxypentonic; C5f Xyloisosaccharinic; C5g 1,4-Anhydro-3-deoxypentitol-2-carboxylic; C6a Anhydroisosaccharinic; C6b Glucometasaccharinic; C6c 3,6-Dideoxyhexonic; C6d 2,5,6-Trihydroxy-3-hexenoic; C6e Glucoisosaccharinic. Dicarboxylic acids: C2c Oxalic; C3d Malonic; C3e Tartronic; C4i Malic; C4j 2-C-Methytartronic; C4k Succinic; C5h 2-Hydroxyglutaric; C5i Hydroxymethylsuccinic; C5j 2,3-Dideoxypentaric; C6f 2-Hydroxyadipic; C6g 2,5-Dihydroxyadipic; C6h 3,4-Dideoxyhexaric

	C1	C2a	C2b	C3a	C3b	C3c	C4a	C4b	C4c	C4d	C4e	C4f	C4g	C4h	C5a	C5b	C5c	C5d	C5e	C5f	C5g	C6a	C6b	C6c	C6d	C6e	C2c	C3d	C3e	C4i	C4j	C4k	C5h	C5i	C5j	C6f	C6g	C6h	REF
Raw material Reaction conditions Temperature (°C) (Reaction time)	Vola-tile acids		Hydroxy acids																								Dicarboxylic acids												
Pine Charge of active alkali: 22% Sulphidity 33% 170 °C	x	x	x		x		x		x						x		x		x	x		x	x			x	x	x		x	x	x	x	x		x	x		36
Pine Charge of active alkali: 18% Sulphidity 25% ca. 170 °C (3.5 h)	x	x	x		x		x								x				x	x						x													32
Pine, spruce Charge of active alkali: 22% Sulphidity 33% ca. 160 °C (3 h)	x	x	x		x			x						x	x	x			x	x		x		x		x	x		x	x	x	x			x	x		x	33

	REF	C1	C2a	C2b	C3a	C3b	C4a	C3c	C4b	C4c	C4d	C4e	C4f	C4g	C4h	C5a	C5b	C5c	C5d	C5e	C5f	C5g	C6a	C6b	C6c	C6d	C6e	C2c	C3d	C3e	C4i	C4j	C4k	C5h	C5i	C5j	C6f	C6g	C6h
Birch Charge of active alkali: 18% Sulphidity 30% ca. 160 °C (4 h)	32	×		×		×	×									×				×	×						×												
Cellulose, starch 1N NaOH 180–300 °C	37	×	×	×		×	×		×								×																					
Cellulose, starch 16N NaOH 240 °C (25 min)	37, 38	×	×	×		×	×		×								×											×					×						
Manman 0.4 M NaOH 97 °C (5 h)	39	×	×			×				×						×						×		×			×											
Manman 0.5 M NaOH 95 °C (2.5 h) 135 °C (2.5 h)	40	×	×	×		×	×			×		×							×			×	×			×	×		×	×	×	×			×	×		
Xylan 0.5 M NaOH 140 °C (2 h)	41			×		×	×												×		×			×													
Xylan 1% NaOH 95 °C (3 h)	42	×		×		×			×											×	×						×											
Glucose 50% NaOH 40–54 °C (1.5 h) 67–112 °C (1.5 h)	43			×	×	×			×										×					×														
Glucose 0.1 M $Ca(OH)_2$ 100 °C (2 h)	44	×	×	×	×	×			×	×	×		×	×		×			×																	×		
Glucose 0.05 M NaOH ca. 100 °C (1 h)	45	×	×	×	×	×		×		×	×													×														

REF	46	45	47	48	49
C6h					
C6g					
C6f					
C5j					
C5i					
C5h					
C4k					
C4j					
C4i					
C3e					
C3d					
C2c					
C6e					
C6d					
C6c					
C6b	×			×	
C6a					
C5g					
C5f				×	
C5e	×			×	
C5d					
C5c					
C5b					
C5a				×	
C4h	×				
C4g					
C4f					
C4e					
C4d					
C4c		×		×	
C4b					
C4a				×	
C3c		×			
C3b	×	×	×	×	×
C3a	×	×	×	×	
C2b		×		×	×
C2a		×			×
C1		×			×
	Glucose 0.099 M NaOH 25 °C, 45 °C	Arabinose 0.05 M NaOH (1 h)	Rhamnose KOH 25, 50, 75 °C	Xylose 0.1 M $Ca(OH)_2$ 100 °C (2 h)	Xylose 0.1 M NaOH 79 °C (20 min)

4. Oxygen-alkali based processes

Molecular oxygen is a suitable oxidizing agent for many purposes. Oxygen has a strong tendency to oxidize organic substances under alkaline conditions. Oxygen-alkali treatment has been applied to the bleaching sequence in order to increase pulp brightness by removing residual lignin. Oxygen alkali delignification can also be used as a pre-treatment of lignocellulosic material for production of bioethanol or other bio based chemicals [17].

The degree of delignification at the termination of Kraft cooking is often around 90%, but may depend on the pulping conditions. After cooking, Kraft pulps are exposed to an oxygen delignification process where a substantial fraction of the residual lignin present in unbleached pulp is removed. When compared to chlorine based delignification, the benefits of oxygen alkali bleaching include lower chemical requirements, higher brightness of pulp, lower water consumption, subsequent effluent discharge and reduced pollution load due to the greater recycling potential of oxygen stage effluents [50]. The drawback of oxygen-alkali delignification is the low yield, approximately 50% delignification, and low selectivity, since carbohydrates are also degraded during these processes. Cellulose depolymerisation causes a deterioration of pulp viscosity and strength characteristics [51].

Typically, oxygen alkali delignification is carried out at a pressure of 0.5–0.75 MPa and at a temperature of 90–110°C with a mass consistency of 10% [13, 24]. Wet oxidation operates with water and oxygen, or air, at elevated temperatures, 150–200 °C, and pressure. The wet oxidation process has been found to convert many organic polymers to oxidized compounds, such as low molecular weight carboxylic acids, or even to CO_2 and H_2O [52, 53].

Alkaline wet oxidation is a variation of wet oxidation. Typically, it has been used as a pre-treatment of herbaceous crops with a low lignin content [52]. Thomsen and Schmidt used alkaline wet oxidation for the fractionation of wheat straw [54]. Lignin content is one of the major issues in biomass enzymatic digestibility, however few studies concerning the application of alkaline wet oxidation to the fractionation of wood meal can be found [55, 56, 57]. The high-lignin biomass has lower fractionation capability and is less responsive to pre-treatment than low-lignin biomass. In addition, the structural changes of the raw material, such as degree of polymerization, increased pore volume, increased surface area and decreased particle size may affect the fractionation [55, 56].

In alkaline wet oxidation, the common bases sodium hydroxide, sodium carbonate and calcium hydroxide are used. Typical reaction conditions include the temperature range 150–200 °C, oxygen pressure of 0.3–1.4 MPa and reaction time of 15 min–6 h [14, 57].

Comparison of alkaline treatment to wet oxidation, where the lignocellulosic material is treated with water and oxygen, showed that the presence of alkali enhances cellulose and hemicellulose convertibility. The benefit of alkaline wet oxidation is the absence of furfural, which is recognized as an inhibitor of enzyme action in a subsequent fermentation step [54]. Another positive effect of alkaline wet oxidation pre-treatment is its ability to remove acetyl and various uronic acid substitutions on hemicellulose, which reduces the accessibility of hemicellulose and cellulose to enzymes [58].

A study of the degradation products of lignocellulosic material treated with alkaline wet oxidation reveals that, among other products, a substantial amount of carboxylic acids can be found. The main acidic degradation products have been reported to be formic, acetic, glycolic, malic, maleic, succinic, oxalic and succinic acids [54, 57, 59].

4.1 Degradation reactions of lignin during oxygen alkali delignification

Oxygen has a biradicalic nature and, therefore, oxidation of lignin in alkaline medium can proceed through either radical processes or ionic reactions. The chemistry of oxygen alkali delignification includes a vast set of reactions involving molecular oxygen, perhydroxyl / superoxide anion radical ($HO_2^{\bullet}/O_2^{\bullet-}$), hydrogen peroxide/ hydroperoxide anion (H_2O_2 / HOO^-) and hydroxyl radical / oxyl-anion radical ($HO^{\bullet}/O^{\bullet-}$) with the phenyl propane units, conjugated phenols, and etherified non-phenolic structures and with their reaction products [51, 60, 61]. The reactions of lignin under oxygen alkali delignification conditions fall into four main categories:

1) Reactions involving phenolates and phenoxy radicals leading to the decomposition of the aromatic ring,
2) degradation of the aliphatic side chain,
3) formation of chromophoric quinone structures, and
4) degradation of aliphatic organic compounds, leading ultimately to the formation of small carboxylic acids, methanol and carbon dioxide [51, 61].

Strongly alkaline conditions are needed in order to ionize free hydroxyl groups of lignin. The primary reaction has been proposed to proceed via phenoxy radical [24]. The phenoxy radical reacts with a superoxide anion radical ($O_2^{\bullet-}$) to form either an oxirane structure or muconic acid structure (Figure 9) [61].

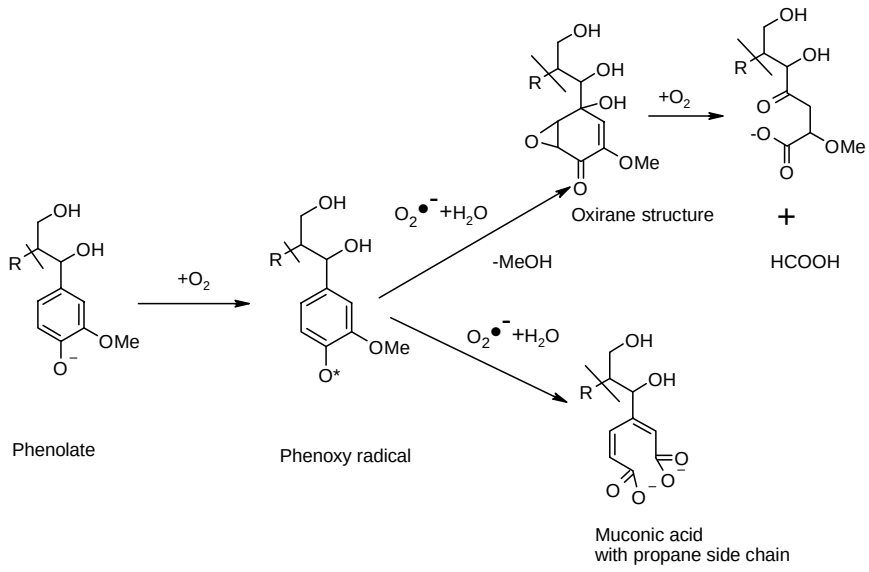

Figure 9. Alkaline oxidation reactions involving phenolates and phenoxy radicals. Modified from ref. [61].

An alkaline environment and elevated temperature promote oxidation of the side chain structures of lignin. Reaction is initiated by the attack of an oxyl-anion radical ($O^{\bullet -}$) to an α-hydroxyl group in an aliphatic side chain of lignin. The reaction proceeds to the elimination of the side chain and to the production of glycolic acid [62]. Among the hydroxy acids, glycolic acid is recognised as typical oxidation product of carbohydrates [63], but it can also be formed by the oxidation of α- and β-conjugated side chain structures of lignin macromolecule as presented (Figure 10). The phenolate structure reacts with oxygen to give an anionic vanillin intermediate. This intermediate reacts further with oxygen, and *p*-quinone is formed, together with a formate anion. Reaction of *p*-quinone with an hydroperoxide anion (HOO^-) leads to the degradation of the aromatic ring and the formation of acetic and maleic acids [61] (Figure 10).

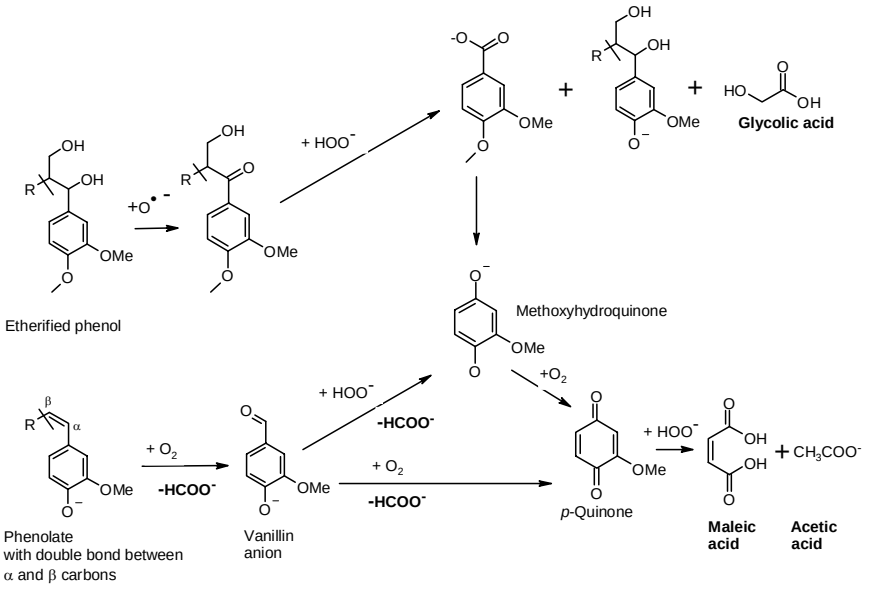

Figure 10. Reactions involving side chain. Modified from ref. [61].

When oxygen reacts with a phenolate anion in the presence of a hydroxyl anion (OH^-), a complex reaction cascade, which includes demethylation, rearrangement and ring re-aromatisation, leads into the formation of catechols. Catechols are easily oxidised to *o*-quinones. By the action of a hydroxyl anion, hydroperoxide anion (HOO^-), and molecular oxygen the aromatic ring is destroyed and aliphatic anionic products resembling muconic acid are formed [51, 61, 64] (Figure 11).

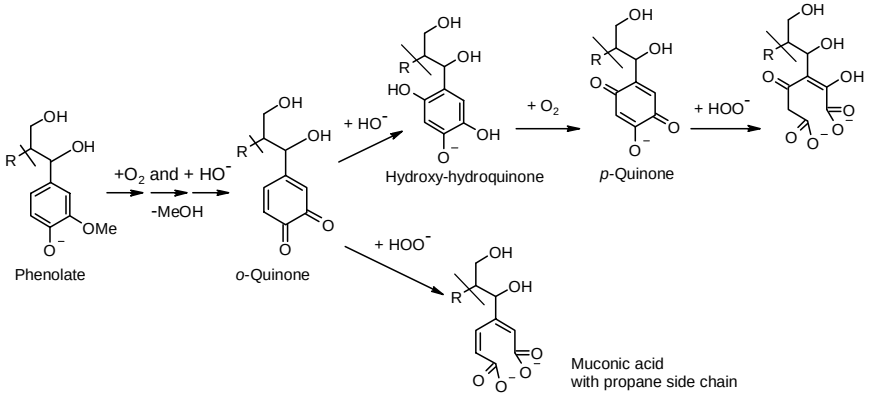

Figure 11. Reactions generating o-quinone structures and their destruction by ring opening. Modified from ref. [61].

Muconic acid and similar structures are the key intermediates in the formation of dicarboxylic aliphatic acids. It may be directly oxidised to four carbon-containing maleic and fumaric acids, and two-carbon oxalic acid [61, 65, 66]. These acids are further degraded to unsaturated dicarboxylic acids, such as succinic and malonic acids, and volatile monocarboxylic acids, acetic and formic acids. In addition, carbon dioxide is also formed. Oxygen, hydroxyl radical, oxyl-anion radical, and hydroperoxide anion are considered the main oxidants in these degradation reactions (Figure 12).

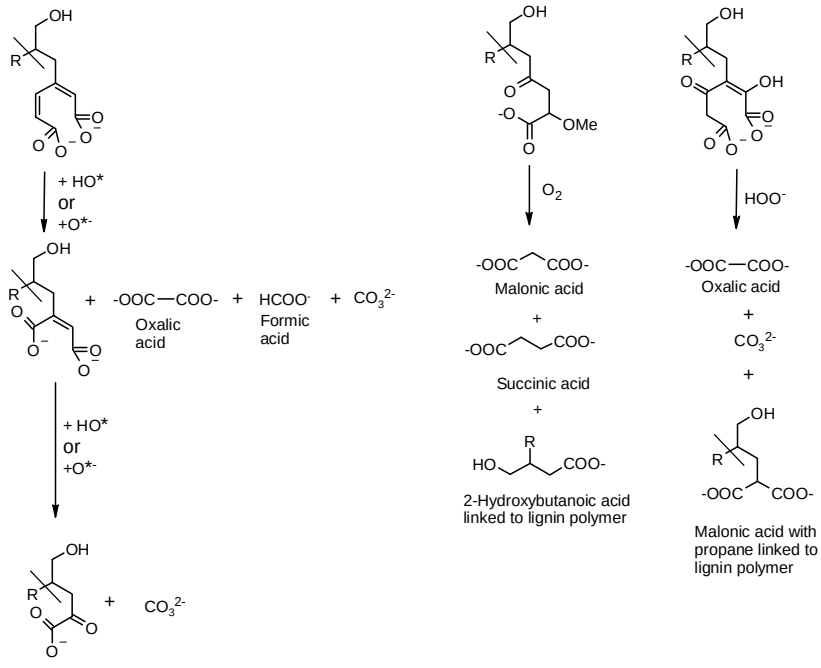

Figure 12. Degradation of aliphatic organic compounds. Modified from ref. [61].

4.2 Degradation reactions of polysaccharides during oxygen alkali delignification

Polysaccharides are also degraded during oxygen alkali delignification. Degradation of polysaccharide chains increases as delignification proceeds [67]. As in Kraft cooking, the degradation occurs through end-wise peeling and random chain cleavage reactions. The C(2) and C(3) positions of the carbohydrate unit in cellulose and hemicelluloses are the most vulnerable reaction points, as they are easily oxidized to a carbonyl group [68]. The formation of a carbonyl group leads to the formation of an alkali-labile glycosidic bond and depolymerisation occurs due the β-alkoxy elimination at C(4) [13]. The hydroxyl radical ($HO^{\bullet}$) is the key oxygen species involved in carbohydrate oxidation [69].

Peeling reactions of the reducing end groups of cellulose and glucomannan lead to extensive acid formation. The hydrolytic and oxidative fragmentations produce formic acid and a wide selection of C(2)-C(6) hydroxy acids. This acid collection resembles the acid pattern obtained under non-oxidative conditions in Kraft cooking [24]. Xylan is degraded in a similar manner to hexose oligosaccharides. However, the degradation of xylo-oligosaccharides is more extensive, especially in the presence of oxygen, than that of cello and manno analogues [70]. Due to the peeling processes, approximately 2-3% yield loss of the Kraft pulp is caused.

The degradation of polysaccharide chain is terminated when the reducing end group is converted to aldonic acid end residue [68, 70]. The stabilization of cellulose and glucomannan chain hexose end groups occurs via β-hydroxy elimination to a glucosone (aldos-2-ulose) intermediate, which is converted to a mannonic acid end group through benzilic acid rearrangement. The glucosone could be further fragmented and oxidized to the arabinonic and erythronic acid end groups [71] (Figure 13). According to Malinen [70], the stabilized arabinonic end-group can further be degraded under an oxygen atmosphere, but this route is not significant under typical oxygen delignification conditions. An analogous reaction cascade occurs with the xylan chain. Here, the intermediate compounds are pentosulose and xylosone and the resulted end groups are lyxonic acid, threonic acid and glyceric acid [42, 70, 71].

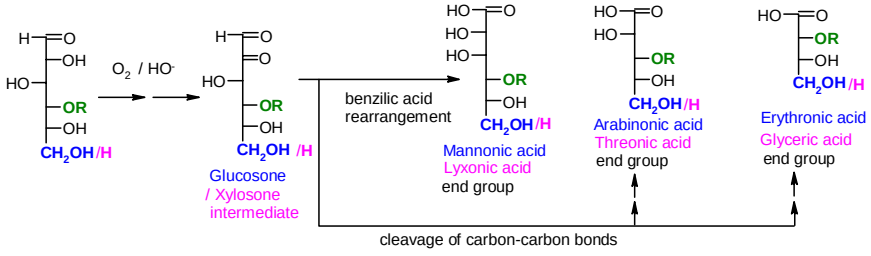

Figure 13. Oxidative stabilization of cellulose (blue font at C(6)) through formation of aldonic acid end group. Stabilization of xylan is shown in magenta coloured font at C(6). Modified from refs [13, 70].

The peeling reactions also occur in alkaline oxidation conditions. By the action of oxygen, the diulose intermediates are converted to formic acid and various C(2)–C(4) hydroxy acids (Figure 14).

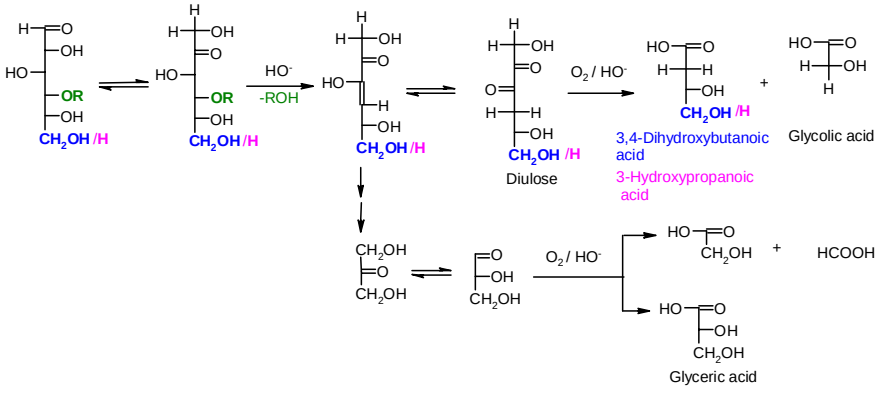

Figure 14. Peeling reactions of cellulose (blue font at C(6)), glucomannan (blue font at C(6)), and xylan (magenta font at C(6)) under oxygen alkali conditions [13].

In summary, carbohydrate degradation takes place both in high-temperature non-reducing environments by the action of alkali and in oxidative conditions present in oxygen alkali delignification stage performed at lower temperature. Similar carboxylic acid mixtures can be found after both treatments, although the mutual ratios of acids varied (Table 4 and Table 5). The main degradation products are non-volatile hydroxy acids, but volatile acids, formic and acetic acids, can also be found. In addition, as well as in Kraft cooking, in the oxygen delignification stage, acetic acid is derived from the splitting of acetyl groups.

Table 5. Degradation of softwood and hardwood, wood polysaccharides and monosaccharides related to wood polysaccharides under various oxygen alkali conditions. The most abundant aliphatic carboxylic acids are grouped as volatile acids, hydroxy acids, and dicarboxylic acids.

Acids: Volatile acids: C1 Formic; C2a Acetic. Hydroxy acids: C2b Glycolic; C3a Glyceric; C3b Deoxyglyceric; C3c Lactic; C3d 3-Hydroxypropanoic; C4a 2-Hydroxybutanoic; C4b 2,4-Dihydroxybutanoic; C4c 3,4-Dihydroxybutanoic; C4d Erythronic; C4e Threonic; C4f Deoxytetronic; C5a 3-Deoxypentonic; C5b 2,3-Dihydroxypentanoic; C5c Xyloisosaccharinic; C5d 1,4-Anhydro-3-deoxypentitol-2-carboxylic acid; C6a Anhydroisosaccharinic; C6b Glucometasaccharinic; C6c 3,6-Dideoxyhexonic; C6d Glucoisosaccharinic. Dicarboxylic acids: C2c Oxalic; C3e Malonic; C3f Tartronic; C4g Malic; C4h Maleic; C4i Fumaric; C4j Succinic; C5e Deoxypentaric; C5f 2-Hydroxyglutaric; C5g 2,3-Dideoxypentaric; C6e C-(2-hydroxyethyl)tartronic; C6f 2-Hydroxyadipic; C6g 2,5-Dihydroxyadipic

	C1	C2a	C2b	C3a	C3b	C3c	C3d	C4a	C4b	C4c	C4d	C4e	C4f	C5a	C5b	C5c	C5d	C6a	C6b	C6c	C6d	C2c	C3e	C3f	C4g	C4h	C4i	C4j	C5e	C5f	C5g	C6e	C6f	C6g	REF
Raw material Reaction conditions Temperature (°C) (Reaction time)			Hydroxy acids																			Dicarboxylic acids														
Pine Kraft pulp Birch Kraft pulp Charge of active alkali: 3% O_2: 0.9 MPa, 120 °C	x	x	x	x	x	x		x	x	x				x		x					x															70
Birch 8.7 gL^{-1} $NaHCO_3$ O_2: 2.0 MPa 135 °C (5 h)	x	x	x	x		x				x	x	x					x					x	x	x	x	x	x	x	x	x		x			72
Cellulose 1M, 3 M NaOH O_2: 0.2, 0.4 MPa 170 °C (3 h) 180 °C (2 h) 190 °C (1 h)	x	x	x			x		x						x	x							x					x			x			x	x	73

REF	71	74	39	39	42	75	76
C6g							
C6f							
C6e							
C5g							
C5f							
C5e							
C4j							
C4i							
C4h							
C4g							
C3f							
C3e							
C2c		×					
C6d		×	×	×	×		
C6c						×	
C6b	×		×				
C6a	×						
C5d				×			
C5c					×	×	
C5b							
C5a	×	×		×	×		
C4f		×					
C4e	×						
C4d	×						
C4c	×		×	×	×	×	×
C4b					×	×	×
C4a					×	×	
C3d					×	×	
C3c	×		×	×	×	×	
C3b							
C3a	×	×		×	×	×	×
C2b	×	×	×	×	×	×	×
C2a						×	
C1	×	×			×	×	×
	Hydrocellulose (cellulose mixed with water) 0.5% NaOH O_2: 0.6 MPa 95 °C (2h)	Hydrocellulose 1 M NaOH 1.1 M H_2O_2 65 °C (4h)	Mannan 0.2 M $NaHCO_3$ O_2: 0.9 MPa 97 °C (2 h)	Mannan 0.2 M NaOH O_2: 0.9 MPa 97 °C (2 h)	Xylan from corn-cob 1% NaOH O_2: 0.5 MPa 95 °C (3 h)	Xylan from birch 1% NaOH O_2: 0.5 MPa 95 °C (3 h)	Arabinose Ribose Xylose 0.833 M KOH 25 °C

REF	77	59, 78	79	80
C6g				
C6f				
C6e				
C5g				
C5f				
C5e				
C4j			×	×
C4i			×	
C4h			×	
C4g			×	
C3f				
C3e				
C2c		×		
C6d				
C6c				
C6b				
C6a				
C5d				
C5c				
C5b				
C5a				
C4f				
C4e				
C4d				
C4c				
C4b			×	
C4a				
C3d				
C3c				
C3b			×	
C3a			×	
C2b	×	×	×	×
C2a	×	×	×	×
C1	×	×	×	×
	Clover, ryegrass 2 gL^{-1} Na_2CO_3 pH 9.3 O_2: 1.2 MPa 175 °C (10 min) 185 °C (10 min) 195 °C (10 min)	Wheat straw 6.5 or 10 gL^{-1} Na_2CO_3 pH 9.3 O_2: 1 or 1.2 MPa 170 °C (10 min) 185 °C (10 min)	Wheat straw 6.5 gL^{-1} Na_2CO_3 pH 9.3 O_2: 1.2 MPa 195 °C (10 min)	Sugarcane bagasse 2 gL^{-1} Na_2CO_3 pH 9.3 O_2: 1.2 MPa 195 °C (15 min)

5. Capillary electrophoresis

Capillary electrophoresis (CE) is a general expression for a wide range of separation techniques, in which a thin capillary serves as a frame for the separation process and an electrical field is applied to move charged species through a liquid or gel. The International Union of Pure and Applied Chemistry (IUPAC) categorises capillary electromigration techniques as follows: capillary electrophoresis (also called capillary zone electrophoresis), capillary gel electrophoresis, capillary isotachophoresis, capillary isoelectric focusing, micellar electrokinetic chromatography, microemulsion electrokinetic chromatography, affinity capillary electrophoresis and capillary electrochromatography [81].

5.1 Principles of separation in capillary zone electrophoresis

In capillary zone electrophoresis (CZE), ions are separated according to their charge-to-size ratio and because of the differences in their electrophoretic mobilities [82]. The effective mobility, μeff of a monovalent weak acid or base is determined by

$$\mu_{eff} = \mu_{act}\,\alpha \qquad (1)$$

where μ_{act} is the actual mobility of the fully charged acid or base and α is the degree of dissociation.

The separation process in CZE, or free solution capillary electrophoresis, takes place in a fused silica capillary. The surface of the untreated capillary becomes negatively charged above pH 2.5 and fully protonated above pH 9 due to the deprotonation of siloxane groups to silanol, SiO^- [83, 84]. The negatively charged surface is covered by positive ions from the buffer, forming an electric double layer. Under the influence of an electric field, the positive ions in the diffuse part of the double layer migrate towards the cathode, resulting in electroosmotic flow, EOF (Figure 15). The shape of the EOF profile is plug-like. Flow velocity is constant over most of the capillary tube cross section. This forms a flat flow profile of electroosmosis, and, therefore, it will add the same velocity component to all solutes, regardless of their radial position. In addition, zone dispersion, e.g. peak broadening is diminished. Many factors affect zone broadening in CZE, including diffusion, Joule heat, electrophoretic dispersion, wall adsorption, injection plug

length and detection width [85]. The electroosmotic mobility (μ_{eo}) depends on the zeta potential (ζ) across the diffuse mobile layer, the immobile Stern layer (η) and the dielectric constant (ε) of the electrolyte [85, 86]:

$$\mu_{eo} = -\zeta\varepsilon/\eta \quad (2)$$

Hence, as the pH increases, the degree of deprotonation of the silanol groups increases, resulting in a high charge density on the capillary wall. In bare fused silica capillaries, the EOF decreases with the square root of the ionic strength, *i.e.* the ionic strength only alters the EOF by shrinking the electrical double layer [84]. The velocity of electroosmosis (υ_{eo}) is given by the Helmholtz-Smoluchowski equation [87]:

$$\upsilon_{eo} = -\zeta\,\varepsilon\,E/\eta = \mu_{eo}\,E \quad (3)$$

where μ_{eo} is the electroosmotic mobility and E the electric field strength. The velocity is proportional to the electric field strength.

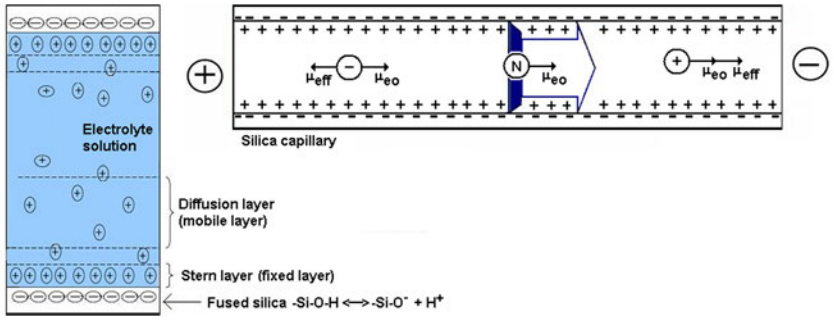

Figure 15. Cross section of fused silica capillary filled with electrolyte solution above pH 2.5. The position of the Stern layer and diffusion layer (left). Factors affecting the apparent mobilities of different analyte species (right) [88].

The apparent mobility (μ_{app}) of an ion is the vector sum of the ion´s effective mobility and the electroosmotic flow (μ_{eo}) (Figure 15):

$$\mu_{app} = \mu_{eff} + \mu_{eo} \quad (4)$$

Neutral compounds migrate in the same direction and at the same velocity as the EOF and they are not separated. Cations move in the same direction as the electroosmotic flow, thus μ_{eff} and μ_{eo} have the same sign and $\mu_{app} > \mu_{eo}$. Anions will move in the opposite direction to the EOF, and for anions, μ_{eff} and μ_{eo} have opposite signs. In the presence of electroosmotic flow, the migration velocity and total migration time of the ion are given by [88]:

$$\upsilon = (\mu_{eff} + \mu_{eo})E \quad (5)$$

and

$$t_m = \frac{L_{\text{det}}\,L_{\text{tot}}}{(\mu_{\text{eff}} + \mu_{\text{eo}})V} \quad (6)$$

where L_{det} and L_{tot} define the capillary length to detection point and the total capillary length, respectively.

The separation voltage applied across the capillary generates current and, due to the resistance of the electrolyte solution, electrical energy is partially converted to heat. This phenomenon is called Joule heating. The temperature is higher in the central region than near the tube wall and therefore the temperature variation across the capillary is parabolic [88]. The temperature difference, ΔT, can be calculated using equation 7:

$$\Delta T \sim E^2 \kappa r^2 \quad (7)$$

where κ is the conductivity of the solution and r the capillary radius. The temperature difference has several negative effects. It leads to band broadening (changes in the migration time due the variation of viscosity) and affects the resolution and the migration profile. To avoid these effects, an effective temperature controlling system is needed to remove the generated heat and to maintain a stable and reproducible column temperature [89].

5.2 Sample pre-treatment for monosaccharide and aliphatic carboxylic acid analyses

In its simplest form, pre-treatment prior to analysis of carbohydrates or aliphatic carboxylic acids includes dilution and filtration to remove interferes. This procedure is suitable for a few sample types, such as beverages, pharmaceuticals and food. For complex samples, such as black liquor, an extended sample pre-treatment, including clean-up and fractionation steps, is needed to isolate fractions of target compounds [90, 91].

Determination of the complete carbohydrate composition and amounts of different carbohydrates is important in respect of process monitoring and product quality. Analysis of monosaccharides in wood derived samples involves cleavage of glycosidic bond and analysis of the constituent carbohydrate residues.

The most common depolymerisation step is two-stage acid hydrolysis. It is carried out using strong sulphuric acid at different concentrations and using various thermal conditions [92, 93, 94]. Unfortunately, the susceptibility of different glycosidic bonds towards acid hydrolysis varies. In particular, glucuronosyl linkages are cleaved incompletely by acid hydrolysis. In addition, sulphuric acid treatment may destroy the released monosaccharides. Therefore, hydrolysis methods have been developed for the depolymerisation of polysaccharides [93, 94].

Acid methanolysis is carried out using 1 M or 2 M hydrochloric acid in methanol mixture. Depolymerisation is performed as a single step at elevated temperature, 85 °C or 100 °C. Acid methanolysis releases both neutral and acidic carbohydrate units from hemicelluloses. Monosaccharides are converted to methyl glycosides, whereas the carboxyl groups of uronic acids are esterified with methyl groups [95].

Enzymatic hydrolysis is an another established approach to determining the structure of polysaccharides [96, 97]. The polysaccharides are fragmented by the

action of mixture of commercial available cellulases and hemicellulases, such as endo-1,4-β-glucanase, β-xylosidase, and α-glucuronidases [93, 94, 96, 97].

Sample pre-treatment for the analysis of aliphatic carboxylic acid is usually quite simple, even with a complex sample matrix, only dilution and filtration is needed [98]. On the other hand, sample treatment using cation exchange resin in either a H^+ or NH_4^+ form may be needed to remove lignin and polysaccharide residues prior to derivatisation and gas chromatographic analysis [99, 100].

5.3 Capillary zone electrophoresis of carbohydrates

Two different methods in capillary electrophoresis are used to separate and determine neutral monosaccharides. First, capillary zone electrophoresis with positive polarity, *i.e.* separation voltage is from anode to cathode [101, 102], and secondly, the method in which polarity is reversed and an electroosmotic modifier is added to the electrolyte to speed up the separation [10, 103]. In both methods, the pH of the electrolyte solution is adjusted above pH 12. In positive polarity mode, the electroosmotic flow dominates and prevents anionic carbohydrate species with low mobilities from migrating out of the capillary. Carbohydrates emerge for detection in the order of increasing effective mobilities [104]. In the reversed-polarity mode, migration times of the anionic compounds are reduced by the co-directional movement of the electroosmotic flow and by the migration of other ionic compounds. CZE with negative polarity is often chosen when the target compounds include a mixture of high mobility anions, such as inorganic anions and carboxylic acids, and/or carbohydrates or when the separation time is intended to shorten [10].

Indirect absorption measurement is a universal and a non-selective monitoring mode for the detection of non-UV absorbing compounds. An absorbing additive of the same charge as the separands, called the probe or chromophore is added to the background electrolyte solution (BGE). The probe is displaced by analyte ions, resulting in negative peaks relative to the high baseline [105]. For the best performance, indirect UV detection requires a probe with the following properties: high molar absorptivity, mobility closely matching that of analytes, migration time stability and good buffering capacity [106, 107]. In the analysis of neutral carbohydrates with indirect UV detection, several probes are used. Examples are 2,6-pyridinedicarboxylic acid (2,6-PDC), which was first applied in the indirect detection of inorganic anions and organic acid [10, 104], sorbic acid [108, 109], 1-naphthylacetic acid (NAA) [9], riboflavin [108, 110], benzoic acid [111], glycylglycine [112] and *p*-nitrophenol [113]. Typical detection limits of neutral carbohydrates, using indirect detection, are 40–200 µM, when either sorbic acid or 2,6-PDC were used as probe [10, 114].

5.3.1 Direct UV detection in carbohydrate analysis

Direct UV detection methods for monitoring carbohydrates can be carried out using complexation with borate [115] or chelation with a copper(II) cation [116].

Borate ions interact with the *cis*-diol moiety of carbohydrate compounds to form anionic complexes which, in turn, enhance the UV absorptivity of carbohydrates around the wavelength of 195–200 nm [115, 117]. According to Hoffstetter-Kuhn, the sensitivity of the detection became better still by increasing the separation temperature up to 60 °C [115]. Unfortunately, such a low UV wavelength imposes serious restrictions on the choice of the composition of the electrolytes, since many useful additives may absorb extensively at that low UV region [7]. Therefore, the means for the chemical optimisation of the electrolyte may be limited.

Chelation of the carbohydrate with a copper(II) cation facilitates the detection of carbohydrates by direct UV detection. An anionic complex is formed in an in-capillary reaction and it can be detected at 245 nm in ammonia electrolyte. However, the sensitivity of the method is limited with the baseline noise caused by the UV absorptivity of the copper(II) electrolyte [116].

5.3.2 Electrochemical detection methods for carbohydrat

Since carbohydrates lack both a charge and an UV chromophore, several detection strategies have been developed alongside UV light-based detections [7, 118]. Electrochemical techniques have proven to be useful methods for the detection of underivatised carbohydrates. Electrochemical detection of carbohydrate in CE can be divided into two main categories: amperometric and conductometric. Detection is based on an electrochemical reaction at the surface of the working electrode in the detector to keep the cell volumes very small with no loss in sensitivity. The detection point is at the capillary end [105]. Typically, separations of underivatised neutral carbohydrates are carried out in strong alkali electrolytes [101, 102, 119]. The common feature of electrochemical detectors, used in contact detection from the electrolyte solution, is the need for accurate spatial alignment of the electrode. However, the structural needs are not available in commercial CE instruments.

Amperometric detection is based on measurement of the current resulting from the oxidation or reduction of compounds at the surface of the electrode that is influenced by the voltage applied to the flow cell. Two approaches to electrochemical detection are reported to be useful for carbohydrate detection, namely amperometric detection at constant potential [101, 120] and pulsed amperometric detection (PAD) [102]. Typical detection limits of carbohydrates in amperometric detection are at nanomolar levels [102, 119, 120]. Amperometric detection is non-discriminatory and, therefore, positive responses originating from amino acids and organic acids may confuse peak assignment [114].

Conductivity detection is a routine monitoring method in ion chromatography [121, 122]. The detection principle is based on measuring differences in the conductivity of the analyte zones and the background electrolyte. The measuring unit consists of two electrodes, which can alternately be either in contact with the electrolyte solution or outside the separation channel. The latter is called contactless conductivity detection (CCD). The two research groups of da Silva [123] and Zemann [122] presented the first CCD devices for capillary electrophoretic separations.

The construction of the detection cell is simple and it can be placed freely over the capillary. In addition, it allows the integration of a second detector.

The main applications of the contactless conductivity detector are monitoring inorganic anions and cations in different matrices [121], but carbohydrate analyses can also be found [124, 125, 126, 127]. The intact carbohydrate compounds are separated using an alkaline electrolyte. Jaroš et al. [124] stated that the conductivity signal is proportional to the difference between the mobilities of the compound and the co-ion of the electrolyte solution and slower peaks produce negative peaks. Figure 16 presents an example of simultaneous use of a CCD and UV detector in a commercial CE instrument for the monitoring of neutral carbohydrates. The limit of detection for intact carbohydrates obtained by CCD is similar to indirect UV detection, *i.e.* 10–20 µm [125]. Since the sensitivity of neither amperometric nor conductivity detection depends on path length, very narrow capillaries such as 5–25 µm can be used [119, 124, 127]. Examples of carbohydrate analyses using UV detection and electrochemical detection are compiled in Table 6.

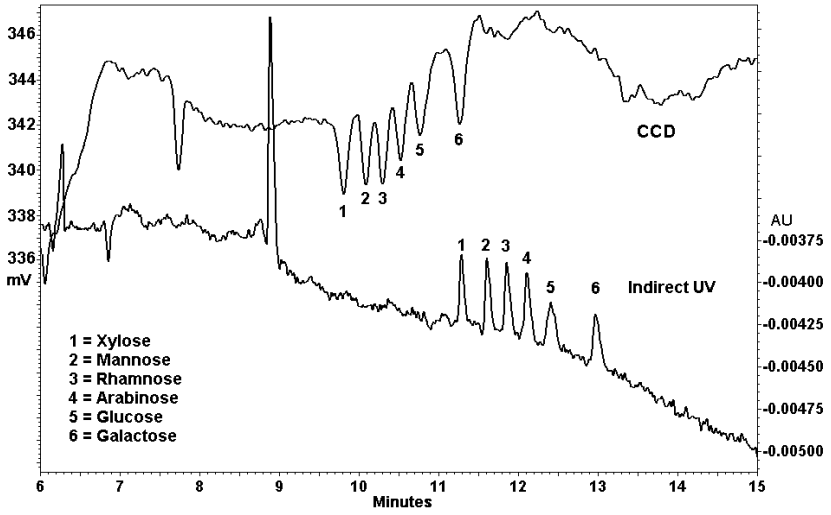

Figure 16. Simultaneous monitoring of six neutral monosaccharides using an in-tandem contactless conductivity detector and UV detector. Monosaccharides: 200 mg L^{-1} each. Separation conditions: 50 mM NaOH, 6 mM potassium sorbate, 0.3 mM myristyltrimethylammonium hydroxide. Separation voltage: -20 kV, injection: 0.5 psi (34.5 mbar) for 8 s, temperature 25 °C, wavelength of indirect UV detection: 254 nm, capillary length (LdetCCD/LdetUV/Ltot): 44/50/60 cm, internal diameter 25 µm [S. Rovio and H. Sirén., unpublished results, 2008].

5.3.3 Precolumn derivatisation for detection of carbohydrates

In order to obtain ionic and UV detectable species of neutral carbohydrates, they are converted either to ionic or UV absorbing compounds prior to analysis. The

most frequently used method is reductive amination [128]. Typical derivatisation agents are 2-aminopyridine (2-AP), 4-aminobenzoic acid ethyl ester (ABEE), 8-aminopyrene-1,3,6-trisulfonate (APTS) and 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) [129]. Derivatisation allows a specific monitoring wavelength to be applied, and electrolyte solutions of moderate alkalinity are usually adequate. It leads to improved resolution and sensitivity, which is especially favourable when laser induced fluorescence (LIF) detection is employed [130].

Table 6. Examples of the analyses of carbohydrates by CE using electrochemical, UV, and LIF detection. Electrophoretic conditions, detection mode, and analytes are listed.

Carbohydrates	**Backgroud electrolyte (BGE)**	**Capillary L_{det}/L_{eff}, diameter**	**Separa-tion voltage**	**Detection, LOD /LOQ(x**	**Ref.**
CEL, FRU, GAL, GLU, LAC, MAN, RHA, RIB, SUC, XYL, stachyose, trehalose, raffinose, lactulose, talose	20 mM / 50 mM / 100 mM NaOH	70 cm, 50 µm	10 kV	amperometric, <50 fmol / NA	101
GLU	10 mM NaOH, 8 mM Na_2CO_3, pH 12	95 cm, 75 µm	25 kV	amperometric, 22.5 fmol (0.9 µM) / NA (xx	102
ARA, FRU, GLU, RHA, XYL, inositol, sorbitol, maltose,	100 mM NaOH	60 cm, 10 µm	30 kV	amperometric, <1.2 fmol (<1 µM) / 1 µM	119
FRU, GLU, LAC, RIB, SUC	100 mM NaOH	80 cm, 25 µm	25 kV	amperometric, <1 fmol (1 µM) / 1 µM	120
Oligosaccharides, dextrin 15, dextrans (starch hydrolysate)	100 mM NaOH, 10 mM CTAB	80 cm, 25 µm	-10 kV	amperometric, <fmol (<1 µM) / >0.8 µM	103
FRU, GLU, SUC, mannitol, in *Ligustrum lucidum* Ait. FRU, GLU, SUC in Moutan Cortex	75 mM NaOH	40 cm, 25 µm	12 kV	amperometric, <2 µM / 2.5 mM	131, 132
FRU, GLU, SUC	30 mM NaOH	70 cm, 25 µm	17 kV	amperometric, <0.6 µM / 1 µM	133
FRU, GLU, SUC, maltose in rice flour	50 mM NaOH	60 cm, 25 µm	16kV	amperometric, 0.6 μgL^{-1}/1 μgL^{-1}	134
FRU, GAL, GLU, SUC	10 mM NaOH, 4.5 mM Na_2HPO_4, 0.2 mM CTAB	35.5 / 44 cm, 20 µm	-25 kV	contactless conductivity detection (CCD),<31 µM / 1 mM	125
GAL, GLU, FUC, MAN, SUC	20 mM 2,6-PDC, 0.5 mM tetradecyltri-methilammonium hydroxide (TTAOH), pH 12.1 (adjusted with NaOH)	88 / 96.5 cm, 30 µm	-30 kV	CCD, NA / NA	124

FRU, GLU, SUC, maltose	12 mM sorbate, 63 mM NaOH or 12 mM riboflavin, 63 mM LiOH	90 / 110 cm, 25 µm	25 kV	256 nm (sorbate), 267 nm (riboflavin) indirect UV, 0.05 mM / NA	110
FRU, GLU, LAC, RHA, RIB, SUC maltose, gluconic acid	2 mM l-naphthylacetic acid (NAA), pH 12.2 (NaOH)	113 / 120 cm, 50 µm	25 kV	222 nm, indirect UV, 0.1 mM / 0.3 mM	9
RIB, MAN, FRU, RHA, GLU, GAL, FUC, SUC, mannitol, sorbitol, pinitol, inositol in leaves of *Banksia grandis* Willd., phloem sap from *E. Globulus*	10 mM / 20 mM benzoate, 0.5 mM myristyltrimethylammonium bromide (MTAB), pH 12.0	31.4 / 36 cm, 50 µm	-20 kV, -25 kV	225 nm, indirect UV, 0.03 mM / 0.1 mM	111
FRU, GAL, GLU, RHA, SUC, sugar acids	6 mM sorbate, 0.001% (w/v) hexadimethrine bromide, pH 12.1 + 5% (v/v) acetone	24.5 / 32 cm, 50 µm	-10 kV	254 nm, indirect UV, 20 µM / 100 µM	135
FRU, GAL, GLU, SUC	6 mM sorbic acid, 10 mM Na_2HPO_4, pH 12.6 (NaOH)	25 / 40 cm, 50 µm	15 kV	260 nm, indirect UV, NA / NA	136
GAL, GLU, MAN, RHA, raffinose, 2-deoxy-D-ribose, sugar acids	6 mM sorbate, 0.001% hexadimethrinebromide, pH 12.2	7.5 / 50 cm, 50 µm	20 kV	254 nm, indirect UV, NA / NA	137
CEL, FRU, GLU, RHA, XYL, lactulose, millibiose, mannitol	6 mM sorbic acid, 1.25 mM CTAB, 50 mM LiOH, pH 12.5	20 / 27 cm, 50 µm	-5 kV	254 nm, indirect UV, 0.01 gL^{-1} (in urine) / NA	109
Sugar acids, aminosugars, sugar alcohols, mono-, di-, trisaccharides	50 mM NaOH, 1.0 mM tryptophan 50 mM NaOH, 5.0 mM tryptophan	34.5 / 50 cm, 25 µm 32 / 56 cm, 50 µm	7 kV	280 nm, indirect UV, 30 fmol / 0.05 pmol	138
GLU, FRU, SUC, maltotriose, maltose	1 mM 1-naphthylacetic acid, pH 12.5 (NaOH)	113 / 120 cm, 75 µm	25 kV	222 nm, indirect UV, 33.5 mgL^{-1} / 50 mgL^{-1}	139
Mono- and disaccharides, amino sugars and sugar alcohols	20 mM 2,6-PDC, 0.5 mM cetyltrimethylammonium hydroxide (CTAH) or CTAB, pH 12.1 (NaOH)	72 / 80.5 cm, 50 µm 104 / 112.5 cm, 50 µm	-25 kV	detection 350 nm, reference 275 nm, indirect UV, or detection 350 nm, reference 230 nm, indirect UV, 20 µM/100 µM 12 mgL^{-1}/50 mg L^{-1}	10, 104, 140
ARA, CEL, FRU, FUC, GAL, GLU, LAC, MAN, RHA, RIB, SUC, XYL,	50 mM glycylglycine, pH 12 (NaOH)	55 / 63 cm, 50 µm	28 kV	207 nm, indirect UV, 30 µg mL^{-1} / 360 µg mL^{-1}	112

xylitol, mannitol, lyxose, maltose, mannose					
FRU, GLU, SUC	70 mM NaOH, 2 mM naphtol blue-black, 80 mM Na_2HPO_4, pH 12.5	18 / 23 cm, 50 µm	9 kV	570 nm, indirect UV, NA / 0.36 mg mL^{-1}	141
CEL, LAC, SUC, maltose	50 mM tetraborate, pH 9.3	62 / 69, 87 / 94, or 110 / 117 cm, 75 µm	20 kV	195 nm, direct UV, NA / NA	115
GLU, RIB, SUC	6 mM $CuSO_4$, 500 mM NH_3, pH 11.6	50 / 73 cm, 50 µm	25 kV	245 nm, direct UV in-capillary complexation with CU(II), 50 µM / 200 µM	116
Debranched wheat straw	commercial carbohydrate separation gel buffer	40 / 47 cm 50 µm eCAP neutral coated capillary	23.5 kV	LIF, APTS derivate, NA / NA	142
ARA, GAL, GLU, XYL, maltotriose	25 mM lithium acetate, pH 5	40 cm / 62 cm, 75 µm	15 kV	LIF, APTS derivate, NA / NA	143
Mixture of mono- and oligosaccharides	45 0mM boric acid, 300 mM NaOH, pH 9.7	40 / 48.5, 50 µm	28 kV	UV 305 nm, ABEE derivate, NA / 0.1–5 mM	144
Galactoglucomannas (T-GGM) from spruce wood	several borate buffers	40 / 48.5, 50 µm	25 kV	UV 306 nm, ABEE derivate, 2 µg mL^{-1}/ 5 µg mL^{-1}	145
ARA, GAL, GLU, MAN, HexA-xylobiose, methyl-glucuronic acid, glucuronic acid in unbleached or bleached chemical pulps	438 mM H_3BO_3, 300 mM NaOH, pH 11.5	38 / 43, 30 µm	constant power 2500 mW	UV 306 nm, ABEE derivate, NA / 2 µM	93
ARA, CEL, GAL, GLU, MAN, XYL, cellotriose, cellotetraose	450 mM borate, 300 mM NaOH, pH 9.94	40 / 48.5 cm, 20 µm	28 kV	UV 305 nm ABEE derivate, 35 mgL^{-1} / 117 mgL^{-1}	146
Neutral and acidic oligosaccharides from an unbleached birch kraft pulp	420 mM H_3BO_3, 220 mM NaOH, pH 9	38 / 43, 30 µm	21 kV	UV 245 nm, 6-AQ derivate, NA / NA	147
ARA, GAL, GAL, GLU, MAN, RHA, XYL from a totally chlorine-free bleaching plant	500 mM borate buffer, pH 9.5	40 / 48.5 cm, 50 µm	16 kV	UV 286 nm 4-amino-benzonitrile (ABN) derivate, 0.5 mgL^{-1} / 5 mg L^{-1}	148

Carbohydrate enantiomers	200 mM borate, 7.5 mM β-cyclodextrin, pH 8.7	85 / 100 cm, 50 µm	35 kV	UV 475 nm 5-aminonaphthalene-2-sulfonic acid (ANA) derivate, NA / NA	149
Maltooligosaccharides	200 mM borate buffer, 200 mM SDS, pH 8.2 (NaOH)	49 / 71 cm, 50 µm	10 kV	UV 245 nm, 1-phenyl-3-methyl-5-pyrazolone (PMP) derivate, 1 µM / 10 µM	150

[(X] LOD and LOQ at lowest level.
[(XX] mass detection limit (molar detection limit) / NA=not announced.

5.4 Capillary zone electrophoresis of carboxylic acids

The analysis of organic acids and inorganic anions is of interest in many fields of industry and research, hence the demand for a simple and reliable method for the fast determination of anions has increased. Small aliphatic carboxylic acids are water-soluble molecules that are negatively charged at pH values around 3–6 and above. They do not have strongly UV-absorbing chromophores in their structures, and therefore the indirect UV detection is the practical choice [151].

The indirect detection is the first choice among detection methods in the capillary electrophoretic separations of aliphatic carboxylic acids, due their lack of UV absorption. As in the case of carbohydrates, the choice of chromophore depends on the structural features of the analytes. In a typical separation, both inorganic anions and organic acids are target compounds, which means that the best-suited chromophores should have an intermediate electrophoretic mobility compared to high mobility inorganic anions and low mobility organic acids. Suitable chromophores are aromatic acids such as pyromellitic acid [152], phthalic acid [137], 2,3-quinolinic acid [153], and 2,3- and 2,6-pyridinedicarboxylic acids [153, 154]. In addition, inorganic probes, such as chromate and molybdate have been employed. However, according to their mobilities, they are better suited for the capillary electrophoretic analysis of inorganic anions [155].

Contactless conductivity detection (CCD) is another universal detection method for the monitoring of charged species [156]. In contrast to the monitoring of carbohydrates with CCD, detection of carboxylic acids by CCD requires a development of a BGE solution with low ionic strength, and thus low conductivity. A good choice is an electrolyte solution based on the mixture of 2-(*N*-morpholino)ethanesulfonic acid (MES) and histidine (HIS) at a pH value of about 6, modified with an electroosmotic flow additive [157].

Most often, a cationic surfactant such as cetyltrimethylammonium bromide (CTAB) or hexadimethrine bromide has been added to the separation solution in order to reverse the electroosmotic flow. The addition of surfactant improves the separation of anions and shortens the migration time as the anionic compounds co-migrate with EOF [158]. Reversing the electroosmosis necessitates reversing the polarity. In reversed polarity mode, injection is carried out at the cathode, while the detection point is placed near the anode of the separation capillary. Table 7

presents examples of CZE methods and instrumental parameters employed in the analyses of small aliphatic carboxylic acids.

Capillary electrophoresis – mass spectrometer (CE-MS) has proven to be an effective analysis system for the separation of anionic carboxylic acids. However, the method development faces challenges when focussing on the development of a volatile electrolyte solution. In addition, the electroosmotic flow needs adjustment when coated capillaries are used. Moreover, a suitable sheath liquid should be formulated. CE-MS has been applied in the analysis of charged metabolites of mouse hepatic metabolites in the central carbon and energy metabolic pathways [159], in the determination of organic acids in atmospheric particles and cloud water [160] and in the separation of five dicarboxylic acids [161].

Table 7. Examples of CE separation conditions for analysis of aliphatic carboxylic acids.

Carboxylic acids (application)	**BGE**	**Capillary L_{det}/L_{eff}, diameter**	**Separation voltage**	**Detection LOD /LOQ[x]**	**Ref.**
Acetic, citric, fumaric, maleic, malic, malonic, oxalic, phthalic, tartaric, aromatic acids, inorganic anions (soil, plant)	30 mM NaH_2PO_4 sodium, 1.0 mM tetradecyltrimethylammonium bromide (TTAB), pH 6.5 + 20% (v/v) acetonitrile	70.4 / 75 cm, 50 µm	-20 kV	190 nm, direct UV, 1 µM / 10 µM	162
Acetic, citric, citraconic, glycolic, formic, fumaric, furanoic, isocitric, oxalic, lactic, maleic, malic, mesaconic, propionic, quinic, pyroglutamic, succinic, inorganic anion (coffee)	500 mM H_3PO_4, 0.5 mM CTAB, pH 6.25 (NaOH)	57 cm, 50 µm	-10 kV	200 nm, direct UV, 4 µM / 50 µM	163
Sugar acids and their lactones	H_3BO_3, pH 7.1 (NaOH)	50 / 57 cm, 75 µm	20 kV	200 nm, direct UV, NA / NA[xx]	164
Adipic, fumaric, glutaric, oxalic, maleic, malonic, pimelic, suberic, succinic, 1,3,5-benzenetricarboxylic, 1,2,4-benzenetricarboxylic, isophthalic, terephthalic, *o*-phthalic, *cis*- and *trans*- 1,4-cyclohexanedicarboxylic, *cis*- and *trans*-1,2-cyclohexanedicarboxylic (alkyd resin samples)	25 mM $Na_2B_4O_7$, 0.5 mM TTAB, 2 mM β-cyclodextrin, 2.25 mM $Ba(OH)_2$	52 / 60cm, 75 µm	-10 kV	185 nm, direct UV, NA / NA	165
Acetic, citric, formic, fumaric, lactic, malic, oxalic, succinic, tartaric	10 mM $Na_2B_4O_7$, 0.5 mM TTAOH, 10 mgL^{-1} Ca^{2+}, 10 mgL^{-1} Mg^{2+}, pH 9,3	53 / 60 cm, 75 µm	-7 kV	185 nm, indirect UV, 0.2 mgL^{-1}/ 0.5 mgL^{-1}	166

Acetic, butyric, lactic, (corn silage, grass silage)	1) 5 mM sorbic acid, 0.5 mM TTAB, pH 6.0 2) 50 mM NaH_2PO_4, 0.5 mM TTAB, pH 5.6	57 / 65cm, 75 µm	1) -25 kV 2) -20 kV	1) 254 nm, indirect UV 2) 185 nm, direct UV	167
Acetic, acrylic, butyric, citraconic, citric, crotonic, glutaconic, glutaric, glyoxylic, 2-OH-isobutyric, itaconic, meaconic, mesoxalic, methacrylic, puryvic	1) 10 mM phosphate, 5 mM tetraborate, 0.001% w/v) hexadimetrinebromide, pH 3.9 2) 5 mM phthalate, 0.001% w/v) hexadimetrinebromide, pH 3.85	24.5 / 32 cm, 50 µm	1) -27 kV 2) -30 kV	1) 185 nm, direct UV, 2 mgL^{-1}/ NA 2) 254 nm, indirect UV, NA / NA	168
Acetic, formic, glycolic, lactic, succinic (old paper)	5 mM 2,6-PDC, 0.5 mM CTAB, pH 5.6 (NaOH)	50.4 / 61 cm, 75 µm	-25 kV	detection 350 nm, reference 200 nm, indirect UV, 0.06 mgL^{-1} / 0.2 mgL^{-1}	146
Citric, formic, lactic, malic, oxalic, succinic, tartaric, inorganic anions and cations	20 mM 2,6-PDC, 0.5 mM cetyltrimethylammonium hydroxide (CTAH), pH 5.7	104 / 112.5 cm, 50 µm	-30 kV	detection 350 nm, reference 230 nm, indirect UV, 0.9 mgL^{-1} / 10 mgL^{-1}	104
Acetic, citric, formic, fumaric,g lutamic, isocitric, lactic, malic, 2-oxoglutaric, pyruvic, succinic (*Bacillus subtilis* cell extract)	4 mM 2,6-PDC, 0.2 mM CTAB, pH 3.5 + 10% ethylene glycol + 10% acetonitrile	70.6 / 75 cm, 75 µm	-25 kV	200 nm, indirect UV, 0.1 µM / 0.5 µM	169
Acetic, citric, lactic, malic, oxalic, succinic, sugar acids	20 mM 2,6-PDC, 0.5 mM CTAH, pH 12.1 (NaOH)	72 / 80.5 cm, 50 µm 104 / 112.5 cm, 50 µm	-25 kV	UV detection 350 nm, reference 275 nm, indirect UV, 6 mgL^{-1} / 20 mgL^{-1} 12 mgL^{-1} / 50 mgL^{-1}	140, 154
Aliphatic dicarboxylic acids (atmospheric aerosol particles)	1) 4.0 mM 2,3-PyDC, 0.5 mM myristyltrimethylammonium hydroxide (MTAH), pH 10.6 2) 4.0 mM 2,6-PDC, 0.5 mM MTAB, pH 11.0 3) 7.0 mM 2,3-PDC, 0.5 mM MTAB, pH 10.2	50 / 58.5 cm, 50 µm	1) -21 kV for 2,3-PyDC 2) -24 kV for 2,6-PDC 3) -20 kV for 2,3-PDC	1) 288 nm 2) 266 nm 3) 266 nm, all indirect UV, 1 mgL^{-1} / 3 mgL^{-1}	153

Acetic, citric, formic, malic, lactic, succinic, tartaric (wine)	7.5 mM 2,6-PDC, 0.5 mM CTAB, 0.5 mM EDTA, pH 5.6	80 cm, 75 µm	-22 kV	detection 350 nm, reference 210 nm, indirect UV, 29 mgL^{-1} / 100 mgL^{-1}	170
Acetic, arabonic,citric, formic, galactaric, galacturonic, gluconic, glycolic, isocitric, α-ketoglutaric, lactic, malic, maleic, malonic, oxalic, propionic, succinic, xylonic (wheat straw hydrolysate)	20 mM 2,3-PDC, 0.3 mM MTAH, 30 mgL^{-1} Ca^{2+}, 30 mgL^{-1} Mg^{2+}, pH 9 (NH_3) + 10% MeOH	70 / 80 cm, 50 µm	-20 kV	254 nm, indirect UV, 2 mgL^{-1} / 5 mgL^{-1}	171
Acetic, formic, oxalic, inorganic anions (process water of a paperboard mill)	2.25 mM pyromellitic acid, 6.50 mM NaOH, 0.75 mM hexamethonium hydroxide 1.60 mM triethanol amine, pH 7.7	50 / 57cm, 50 µm	-20 kV	254 nm, indirect UV, 0.5 mgL^{-1} / NA	172
Acetic, citric, lactic, malic, oxalic, succinic, tartaric, inorganic anions (beverages)	3 mM pyromellitic acid, 3 mM diethylene triamine (DETA) pH 7.5 (TRIS)	37 / 44 cm, 75 µm	-20 kV	220 nm, indirect UV, 6 μgL^{-1} / 20 μgL^{-1}	173
Acetic, aspartic, citric, glutamic, gluconic, lactic, malic, oxalic, succinic, tartaric (beverages)	1) 5 mM trimellitic acid, 1 mM TTAB, pH 9.0 2) pH 5.5	63 / 70 cm, 75 µm	-20 kV	220 nm, indirect UV, 2 µM/10 µM	174
Acetic, butyric, cyclohexanoic, formic, glycolic, malonic, oxalic, propionic, tartaric, inorganic anions (sour gas)	15 mM trimellitic acid, 200 mM TRIS, hexadimethrine bromide, pH 9.0 + 0.1% polyvinyl alcohol	56 / 64.5 cm, 50 µm	-30 kV	240 nm, indirect UV, 1 mgL^{-1} / 2 mgL^{-1}	175
Acetic, lactic, succinic	15mM sodium benzoate, hexadimetrinebromide, pH 6.22 (1.0M Tris–base buffer), 24% (v/v) MeOH	50 / 60 cm, 75 µm	-20 kV	214 nm, indirect UV, 0.3 μgmL^{-1} / 0.8 μgmL^{-1}	176
Crotonic, citraconic, hydroxybutyric, itaconic, mesaconic, methacrylic, puryvic	5 mM phthalate, 0.001% hexadimethrinebromide, pH 3.85	7.5 / 32 cm, 50 µm	30 kV	185 nm, indirect UV, NA / NA	137
Acetic oxalic, formic, glycolic, malic, succinic, tartaric, (beet sugar related samples)	5 mM phthalate, 2% (v/v) OFM, 0.2 mM Ca^{2+} pH 5.6 (NaOH)	53 / 60 cm, 75 µm	-20 kV	254 nm, indirect UV, NA / 10 mgL^{-1}	177
Acetic, formic, glutaric, glycolic, α-D-isosaccharinic acid lactic (degradation of cellulose)	4 mM nicotinic acid, 15 mM creatinine, pH 5.37	52.4 / 60 cm, 75 µm	25 kV	214 nm, indirect UV, NA / NA	178

Acetic, formic, malonic, oxalic, succinic, inorganic anions (Bayern liquors)	5.0 mM MoO_3, 1.3 mM CTAB, 26.8 mM diethanolamine, pH 9.2	72 / 80 cm, 75 µm	-30 kV	214 nm, indirect UV, 0.3 mgL^{-1} / 1 mgL^{-1}	179
Acetic, formic, inorganic anions	5 mM TRIS buffer, 5 mM molybdate, 0.15 mM CTAH, 0.01% PVA, pH 7.9	65 cm, 75 µm	-20 kV	230 nm, indirect UV, 0.1 mgL^{-1} / 0.4 mgL^{-1}	155
Formic, oxalic, inorganic anions (green, white, black liquors)	10 mM Na_2CrO_4, 2 mM TTAB	72 / 80.5 cm, 75 µm	-25 µA	detection 525 nm, reference 275 nm, indirect UV, NA / 0.5 mgL^{-1}	180
Acetic, citric, glucuronic, lactic, malic, oxalic, pyruvic, succinic, tartaric, ascorbic (aromatic), inorganic anions (juice, wine)	3 mM 1,3,5-benzenetricarboxylic acid (BTA), 15 mM TRIS, 1.5 mM tetraethylenepentamine (TEPA), pH 8.4	65 cm, 50 µm	-25 kV	240 nm, indirect UV, 2 µM / 10 µM	181
Acetic, citric, lactic, malic, succinic, tartaric (wines)	10 mM 3,5-dinitrobenzoic acid, 0.2 mM CTAB, pH 3.6	50 / 57 cm, 75 µm	-25 kV	254 nm, indirect UV, 0.6 mgL^{-1}/ 2 mgL^{-1}	182
29 carboxylic acids, both aliphatic and aromatic	20 mM 2-morpholinoethane sulfonic acid (MES)/NaOH, pH 6.0 + 10% (v/v) MeOH	85 / 92 / 100 cm, 50 µm, polyacrylamide coated capillary	-30 kV	CCD and UV at 220 nm, direct UV, 0.6 µM / 10 µM (CCD)	127
Acetic, citric, formic, fumaric, glycolic, lactic, malic, propionic, pyruvic, succinic, tartaric, aromatic carboxylic acids, inorganic acids (juices, Chinese herbal, plants)	20 mM MES/histidine (His), pH 6.0, 0.1 mM CTAB, 0.025% HP-β-CD, 10% MeOH	42 / 50 cm, 50 µm	-15 kV	CCD, 0.5 mgL^{-1} / 1.5 mgL^{-1}	183
Carboxylic acids and other anionic metabolites	50 mM ammonium acetate solution (pH 8.5) 50 mM acetic acid (pH 3.4)	110 cm, 50 µm COSMO(+), chemically coated with a cationic polymer, capillary	-30 kV	ESI-TOF MS, negative mode, 0.09 µM / 1 µM	159
38 organic acids (atmospheric particles, cloud water)	20 µm ammonium acetate pH 9.1 (NH_4OH) + 10% MeOH	80 cm, 50 µm	30kV	ESI-TOF MS, negative mode, 0.4 nM / 10 nM	160
Glutaric, malic, malonic, succinic	4 mM pyromellitic acid, 4 mM naphthalene disulfonate, 2 mM DETA, 20% methanol	100 cm, 100 µm	-28.5 kV	ESI-MS negative mode, 1 mgL^{-1} / NA	184

(X LOD and LOQ at lowest level.
(XX NA=not announced.

5.5 Chromatographic methods for carbohydrates and aliphatic carboxylic acids

Chromatographic separation techniques offer well-established alternatives for analysing carbohydrates and organic acids of various samples. The main chromatographic systems used for the separation of neutral carbohydrates or aliphatic carboxylic acids are gas chromatography (GC) and high-performance liquid chromatography (HPLC) with several subtechniques [94, 185].

5.5.1 Analysis of carbohydrates and aliphatic carboxylic acids by gas chromatography

As both carbohydrates and carboxylic acids are polar compounds in nature, complicated, and time-consuming, derivatisation is required prior to gas chromatographic analysis. The classical methods for derivatisation of carbohydrates consist of replacing all the active-hydrogen atoms by non-polar substituents. Methyl, trifluoroacetyl, trimethylsilyl and *tert*-butyldimethylsilyl ethers have been the most popular derivatives. The drawback of derivatisation is that these reactions give a different compound for every anomeric form of the carbohydrate, which produces complicated chromatograms when a complex mixture has to be separated [90, 91, 92]. Reduction of aldoses with sodium borohydride eliminates anomeric centres by converting them to alditols. This simplifies the chromatographic profile, since each alditol produces only one peak [91].

Volatile carboxylic acids can be determined as benzyl esters, whereas non-volatile acids, such as hydroxy monocarboxylic and dicarboxylic acids are derivatised with trimethylsilyl reagents [185]. Both derivatised carbohydrates and aliphatic acids are separated in GC using capillary columns coated with a crosslinked non-polar polysiloxane phase. The temperature programming is employed in the range of 60 -300 °C and samples are usually injected using split or splitless modes [3, 186, 187].

Both flame ionisation detector (FID) and mass spectrometric detection (MS) are employed in GC for carbohydrates and carboxylic acids [93, 94, 188, 189, 190, 191]. The first of these is a universal detector and, therefore, model compounds are needed in identifying and quantifying the monitored signals. Mass spectrometric detection provides high detection sensitivity. Quantitation can be done using peak areas obtained from total ion current (TIC) and external calibration. Peak identification can be performed on the basis of fragment ions and by comparing fragmentation patterns to the data in a spectral library [187]. In selected ion monitoring (SIM) mode, the identification and quantitation of the TMS derivatives is measured on the basis of one or two of their main fragment ions [186, 187].

5.5.2 Analysis of carbohydrates and aliphatic carboxylic acids by liquid chromatography

5.5.2.1 Carbohydrates by HPLC

High performance liquid chromatography (HPLC) is a versatile method in the separation of carbohydrate compounds and aliphatic carboxylic acids. Among the liquid chromatographic methods, high-performance anion-exchange chromatograph (HPAEC), equipped with a pulsed amperometric detector (PAD), has been established as a powerful tool for carbohydrate analysis. The separation principle of HPAEC-PAD is based on the fact that carbohydrates are weak acids, which form anions in an eluent of high pH [192]. A standard procedure for the determination of the contents of the five principal, neutral monosaccharides found in wood pulps is described in SCAN-test Method CM-71 [193]. Compounds are separated due the differences in ion exchange affinities of the individual compounds.

Underivatised carbohydrates are separated using polymer-based stationary phases, which have stability over a wide pH range. A typical column used in carbohydrate analyses is CarboPac1, an anion-exchange column that is packed with polystyrene-divinylbenzene particles [194]. Installation of a guard column is necessary in order to protect the analytical column from impurities. The mobile phase composition in HPAEC influences the selectivity and rapidity of separation, as well as the sensitivity of detection. Mono- and disaccharides, alditols, and sugar acids can be separated using a solution of less than 50 mM NaOH as the eluent in isocratic run. Gradient elution is used to improve separations, or to accelerate the elution of late-eluting components [195]. In addition, by applying acetate ion in alkaline solution in gradient elution, the separation of complex mixtures of saccharides with different sizes and acidities will be improved [97, 196].

HPAEC may be interfaced with mass spectrometry, but a desalter must be installed in between these instruments to convert the eluent from the chromatograph into an electrospray ionisation (ESI)-compatible solution [197,198].

Ligand-exchange chromatography (LEC) is the first HPLC variation for the analysis of carbohydrates. In LEC, cation-exchange columns are loaded with metal ions, including calcium, lead and silver, electrostatically immobilised on the surface of polystyrene-divinylbenzene resin. The choice of metal cation effects the selectivity. The retention mechanism is mainly based on the formation of weak complexes between saccharides and metal ions. In addition, elution is affected by size-exclusion mechanisms and oligomers are monitored in order of decreasing molecular mass. Underivatised carbohydrates are separated using water as the eluent. The eluted carbohydrates are monitored either with a pulsed amperometric detector or with a refractive index (RI) detector [8].

5.5.2.2 Aliphatic carboxylic acids by HPLC

Aliphatic carboxylic acids may determined by ion-exchange (IC), ion-exclusion (IEC), and reversed phase (RP) chromatography. Among those, ion-exchange chromatography has gained the broadest acceptance as the tool for these analyses [199]. Similarly, ion-exclusion chromatography is suitable for the separation of a wide range of small, neutral, and partially ionised molecules [200]. Figure 17 shows the difference between the separation principles of these two ion chromatography methods.

In an anion exchange chromatography, ions in solution and functional groups on the stationary phase react by stoichiometric chemical reaction. Typical functional groups of the stationary phase are quaternary ammonium groups bonded in the surface of organic polymer, such as poly(methacrylates), poly(styrenedivinylbenzene, PS-DVB), and poly(vinylalcohol) [201]. The selectivity of anion exchange is affected by the relative sample and eluent charges, eluent concentration and the pH of the mobile phase [199].

In IEC, the stationary phase consists of a completely sulfonated cation exchanger, bonded on the surface of poly(styrenedivinylbenzene), that has sulfonic acids groups and are electrically neutral with protons as counter ions. In aqueous solutions, the functional groups are hydrated, which generates a negatively charged membrane, Donnan membrane, over the hydrated sulfonic acid groups. Only uncharged, non-dissociated molecules, such as water, can pass this virtual membrane. Carboxylic acids can be separated when a strong mineral acid, such as sulfuric acid, is used as the mobile phase. Under highly acidic environments carboxylic acids are present in nondissociated form. They pass through the Donnan membrane while completely dissociated sulfuric acid is excluded. The carboxylic acids are retained on the stationary phase by a combination of ion-exclusion and hydrophobic interactions [199, 200]. The degree of retention depends on the pKa of the individual carboxylic acid, while the dissociation constant determines the amount of protonated fraction of the compound at a given pH of the eluent.

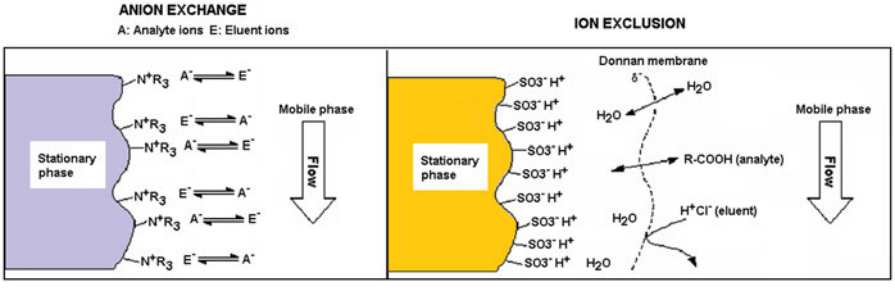

Figure 17. Schematic diagram of the anion exchange process (left) and ion exclusion chromatography based on Donnan exclusion. Modified from ref. [201].

The detection of separated carboxylic acids can be carried out using conductivity detection, which is the most popular detector in ion chromatography [201]. To achieve a sensitive response, chemical suppression is needed when the eluent is a high conductivity solvent. The suppressor is installed between column outlet and detector [199, 202]. UV detection at the wavelength below 210 nm is also used, but the detection sensitivity is rather low, 0.01–10 mM [199]. Refractive index detection (RI) is useful when the compounds have little or no measurable UV detection [186].

Recently, the coupling of an ion chromatograph with a mass spectrometer has been presented by Käkölä et al. [202]. As with conductivity detection, and also with MS, chemical suppression is needed because of nonvolatile eluent [203]. Analysis of aliphatic carboxylic acids is performed using RP-HPLC [98]. They used formic acid as the volatile eluent and the organic acids were monitored in negative ion mode.

6. Aims of the study

1) To develop a CZE method for the analysis of neutral mono-, di-, and oligosaccharides and sugar alcohols based on a separation medium of extreme alkalinity and a direct UV detection. To demonstrate the applicability of the method for the analysis of the carbohydrates in wood based samples. (**I**, **II**.)

2) To develop a CZE method for the analysis of aliphatic carboxylic acids in complex matrices. (**III**, **V**)

3) To evaluate the applicability of the above methods in wood processing, such as

   - kraft cooking (**VI**)
   - oxygen-alkali treatment of lignin as model reaction of oxidative delignification (**IV**)
   - oxygen-alkali treatment of wood as pretreatment for bioethanol production (**V**).

4) To evaluate the role of polysaccharide and lignin reactions as a source of acidic degradation products. (**IV**, **V**)

7. Experimental

7.1 Chemicals and materials

The compounds, and other chemicals and materials used in this work, are listed in Tables 8 and 9. Table 10 compiles the process samples.

Table 8. The compounds used in this work.

Compound / Material	Molecular formula	MW (g/mol)	Purity and Supplier	Paper
Carbohydrates				
Aldopentoses				
D-(-)-Arabinose	$C_5H_{10}O_5$	150.1	98%, J.T. Baker	I, II
D-(-)-Ribose	$C_5H_{10}O_5$	150.1	Hoffman – La Roche[a]	I
D-(+)-Xylose	$C_5H_{10}O_5$	150.1	≥98%, Fluka	I, II
Aldohexoses				
D-(+)-Galactose	$C_6H_{12}O_6$	180.2	≥99%, Fluka	I, II
D-(+)-Glucose	$C_6H_{12}O_6$	180.2	99%, Merck	I, II
D-(+)-Mannose	$C_6H_{12}O_6$	180.2	≥99%, Fluka	I, II
Deoxyhexoses				
D-(+)-Fucose	$C_6H_{12}O_5$	164.2	≥99%, Fluka	I
L-Rhamnose	$C_6H_{12}O_5$	164.2	99%, Sigma	I, II
Ketohexose				
D-(-)-Fructose	$C_6H_{12}O_6$	180.2	99%, Merck	I
Disaccharides				
D-(+)-Cellobiose	$C_{12}H_{22}O_{11}$	324.3	≥99%, Fluka	I
Sucrose	$C_{12}H_{22}O_{11}$	342.3	>98%, Fluka	I
Sugar alcohols				
Mannitol	$C_6H_{14}O_6$	182.2	Merck[a]	I
Xylitol	$C_5H_{12}O_5$	152.2	99%, Merck	I

Carboxylic acids

Acetic acid	CH_3COOH	60.05	99.5%, Fluka	III–V
Citric acid	HOOC-$CH_2C(OH)(COOH)CH_2$-COOH	192.12	99.5%, Fluka, Merck	III
2,5-Dihydroxy-pentanoic acid	$HOOCCH(OH)CH_2CH_2CH_3$	134.13	synthesis product, donation by Klaus Niemelä, VTT	V, VI
D-Erythronic acid	$HOCH_2(CH(OH))_2COOH$	136.10	95%, Aldrich	
Formic acid	HCOOH	46.03	98–100%, Riedel-deHaën, J.T.Baker	III–VI
Fumaric acid	$HOOC(CH)_2COOH$	116.07	99.5%, BDH	III–V
2-Furan carboxylic acid	$C_5H_4O_3$	112.09	Na, salt, ≥98.0%, Fluka	III
Gluconic	$HOCH_2(CHOH)_4COOH$	196.16	≥99%, Sigma	
Glucoisosaccharinic acid (GISA)	$HOCH_2CH(OH)CH_2C(OH)(COOH)CH_2OH$	180.16	Ca-salt, 98%, Alfa Aesar	V, VI
D-Glyceric acid	$HOCH_2CH(OH)COOH$	106.08	99%, Aldrich	
Glycolic acid	$CH_2(OH)COOH$	76.05	99%, Fluka	III–VI
2-Hydroxybutanoicacid	$HOOCCH(OH)CH_2CH_3$	104.1	97%, Aldrich	V, VI
3-Hydroxypropionic acid	$HOOCCH_2CH_2OH$	90.08	Na-salt, 30% in water, TCI	V
DL-Lactic acid	$CH_3CH(OH)COOH$	90.08	88%, BDH	III–V
DL-Malic acid	$HOOCCH_2CH(OH)COOH$	134.09	≥99%, Aldrich	III–V
Maleic acid	HOOCCH=CHCOOH	116.1	Na-salt, 99%, Sigma	III
Malonic acid	$HOOCCH_2COOH$	104.1	99%, Aldrich	III–V
Oxalic acid	HOOC-COOH	90.03	99%, Aldrich	III–VI
Succinic acid	$HOOC(CH_2)_2COOH$	118.09	≥99.5%, Fluka	III–V
Tartaric acid	$HOOC(CHOH)_2COOH$	150.09	Na-salt, >99%, Riedel-deHaën	III
Xyloisosaccharinic acid (XISA)	$HOOCC(CH_2OH)(OH)CH_2CH_2OH$	150.13	synthesis product, donation by Klaus Niemelä, VTT	V, VI
Xylonic acid	$HOCH_2(CH(OH))_3COOH$	166.13	>97%, Carbosynth	

[a] Purity information is not available.

Table 9. Other chemicals and materials used in this work.

Compound	Purpose	Manufacturer / supplier	Paper
Barium chloride ($BaCl_2$•$2H_2O$)	Electrolyte chemical	Merck	III–VI
Cetyltrimethylammonium bromide (CTAB)	Electrolyte chemical	Merck	III–VI
Glacial acetic acid	For capillary conditioning between runs	Merck	I, II
Hydrochloric acid (HCl)	For capillary conditioning between runs	Merck	III–VI
Potassium hydroxide (KOH)	Electrolyte chemical	Merck	I
Sodium carbonate (Na_2CO_3)	Analyte	Merck	III–VI
Sodium hydrogen carbonate ($NaHCO_3$)	Buffer chemical in oxidation	Merck	I, II
Sodium hydroxide (NaOH)	Electrolyte chemical; for pH adjustement	FF-Chemicals, Merck, Akzo Nobel	I–VI
Disodium hydrogen phosphate dihydrate (Na_2HPO_4•$2H_2O$)	Electrolyte chemical	Merck, J.T.Baker	I, II
Sodium phosphate dodecahydrate (Na_3PO_4•$12H_2O$)	Electrolyte chemical; analyte	Merck	I, II, III
Sodium sulphate (Na_2SO_4)	Analyte	Sigma	III–VI
2,3-Pyrazine dicarboxylic acid	Electrolyte chemical	≥98.0%, Fluka	III–VI
Tricine	Electrolyte chemical	BDH, Sigma	III–VI
Triethanolamine	Electrolyte chemical	Sigma	III–VI
Urea	Electrolyte chemical	J.T.Baker, Merck	III–VI
Milli-Q water	Purified water solution	Millipore	I–VI
Disposable filters, GHP Acrodisc 0.45 µm	Filtration of electrolyte solution and samples	Gelman Sciences	I–VI
Fused silica capillary	50 µm i.d., 375 µm o.d.	Teknolab AS	I–VI

Table 10. Samples used in the studies I–VI.

Sample	From	Target analytes	Paper
Orange juice	Local market	Carbohydrates	I
Pineapple juice	Local market	Carbohydrates	I
Lemon juice	Local market	Carbohydrates	I
Alcoholic beverage	Tax-free shop	Carbohydrates	I
Acid hydrolysates of cellulose fiber samples: oat spelt, wheat straw, thermomechanical pulp (TMP) made of spruce, aspen stemwood, and bleached birch Kraft pulp	VTT	Carbohydrates	II
Commercial softwood (SW) and hardwood (HW) lignin, treated under alkaline oxygen delignification conditions	VTT	Carboxylic acids	III, IV
Red wine samples (made with the Pinot Noir grape)	Local liquor store	Carboxylic acids	III
Norway spruce treated with alkaline oxidation, with and without catalyst	VTT, University of Helsinki	Carboxylic acids	V
The black liquor of both softwood and hardwood	Kraft pulp mill	Carboxylic acids	VI

7.1.1 Sample pretreatment prior the CE analyses

The typical sample pretreatment of the samples include dilution and filtration. Liquid samples of lignocellulosic origin were diluted with 20 mM sodium hydroxide. The oxidative treatments of spruce chips and softwood lignin prior to analyses are described in Papers **V** and **IV**, respectively. Figure 18 presents a schematic diagram of oxidation and analyses of both spruce and lignin samples.

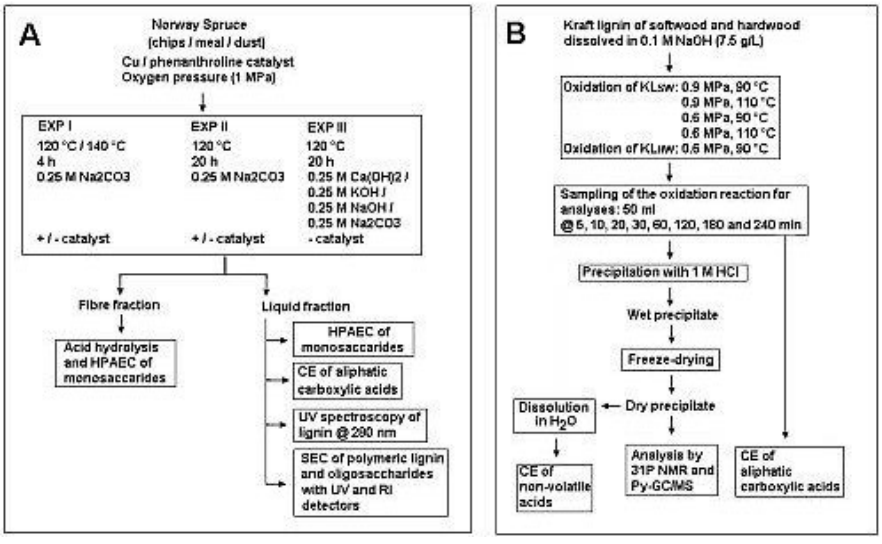

Figure 18. Schematic diagram of oxidation procedure and analyses of spruce samples (A) and lignin samples (B). Detailed descriptions of treatments are presented in Papers V and IV, respectively.

7.1.2 Alkaline oxidation of single monosaccharides

Single monosaccharides were oxidised under mild alkaline conditions. Sodium hydrogen carbonate buffer (1.0 mol L^{-1}, pH 9.5 adjusted with NaOH) was used as a medium. 1.5 g of carbohydrate (D-arabinose, D-galactose, D-glucose, D-mannose and D-xylose) was weighed to 30 mL of sodium hydrogen carbonate buffer. The oxidation was performed at 80 °C under oxygen pressure of 20 bar for 5 hours. The pH was measured after oxidation. Table 11 compiles the experimental parameters kept during oxidation of monosaccharides.

After oxidation, the carboxylic acid content was analysed with the CE method described in Paper **V**. Samples were diluted to 1:50 and 1:100 (v/v) with 20 mM NaOH prior to analysis. The peaks were identified using the standard addition technique and the quantitation was done using an external calibration method.

Table 11. Parameters in alkaline oxidation of monosaccharides.

Carbohydrate	g	Volume (L)	$P(O_2)$ (bar)	Time (h)	Temp. (°C)	Buffer	pH start	pH end	Δ pH
D-Arabinose	1.5	0.03	20.0	5	80	1.0 M	9.5	7.59	1.91
D-Galactose	1.5	0.03	20.0	5	80	1.0 M	9.5	7.76	1.74
D-Glucose	1.55	0.03	20.0	5	80	1.0 M	9.5	7.65	1.85
D-Mannose	1.52	0.03	20.0	5	80	1.0 M	9.5	8.25	1.25
D-Xylose	1.5	0.03	20.0	5	80	1.0 M	9.5	7.59	1.91

7.2 Instruments

7.2.1 Capillary electrophoresis

All capillary electrophoretic analyses in the studies **I–VI** were performed with a P/ACE MDQ (Beckman-Coulter, Fullerton, CA, USA) CE instrument equipped with a photodiode array detector. The controlling software was 32Karat, version 7.0.

All pH measurements were made with a Denver pH meter, Model 20, with combination electrode (Denver Instrument, Denver, CO, USA). The calibration was performed using commercial buffers of pH 7.00 (± 0.01), 10.00 (±0.01), and 12.00 (±0.01) (Reagecon, Shannon, Ireland, and Reagena Oy, Toivala, Finland).

7.2.2 Electrolyte solutions and instrumental parameters for carbohydrate and carboxylic acid analysis

Neutral carbohydrates were analysed using an electrolyte solution consisting of 130 mM NaOH and 36 mM $Na_2HPO_4 \cdot 2H_2O$. The electrolyte solution was prepared by mixing a 450 mM stock solution of disodium hydrogen phosphate dehydrate with 1 M NaOH solution. The measured pH value and the ionic strength of the electrolyte solution were 12.6 and 0.217 M, respectively (**I**, **II**).

Two compositions of the background electrolyte solutions (BGE) for the analysis of the aliphatic carboxylic acids were employed. First, a BGE solution consisted of 20 mM 2,3-PyDC, 65 mM tricine, 2 mM $BaCl_2$, 0.5 mM CTAB and 2 M urea. The pH value of the electrolyte was adjusted to 8.08 with 0.1M NaOH (**III**, **IV**). The second variation of the BGE contained 30 mM tricine and the pH was adjusted to 8.06 (± 0.02) with triethanolamine (**V**, **VI**). Stock solutions of 100 mM 2,3-PDC, tricine, CTAB, and barium chloride were prepared, and a suitable volume of each component was added to the solution, while the appropriate amount of urea was weighed for each portion of the electrolyte. Table 12 compiles the electrolyte compositions and instrumental parameters used in the analyses.

Before measurements, the new capillaries were conditioned by rinsing sequentially with 0.1 M sodium hydroxide, Milli-Q water, and the electrolyte solution, each solution for 20 min.

Table 12. Instrumental parameters used in the analyses of carbohydrates and aliphatic carboxylic acids.

	Electrolyte	Separation voltage (kV)	Injection pressure (psi) and duration	Detection wavelength (nm), detection mode	Temperature (°C), capillary / sample storage	Capillary length (Ldet / Ltot, cm)	Paper
Carbo-hydrates	130 mM NaOH – 36 mM $Na_2HPO_4 \cdot 2H_2O$	+16 +17	0.5 / 4 s 0.5 / 6 s	270, direct	15 / 15 20 / 20	50 / 60	I II
Carboxylic acids	20 mM 2,3-PyDC, 65 mM tricine, 2 mM $BaCl_2$, 0.5 mM CTAB and 2 M urea, pH 8.08 (TEA) (III)	-15	0.5 / 10 s	281, indirect	15 / 20	50 / 60	III,
	20 mM 2,3-PyDC, 30 mM tricine, 2 mM $BaCl_2$, 0.5 mM CTAB and 2 M urea, pH 8.06 (TEA) (IV, V)		0.5 /20 s		15 / 15	50/60 100/110	IV, V

7.2.3 Other instruments

Other instrumental analysis methods were also applied in this study. Detailed descriptions of instrumental parameter can be found in Papers **II**, **IV** and **V**.

A Dionex anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used for the monosaccharide analysis (**II**, **V**). The content of the soluble lignin was measured using a UV spectrophotometer (**V**). More detailed descriptions of the experimental conditions are given in Papers **I–VI**.

8. Results and discussion

8.1 Method for the carbohydrate separation

The most common detector in commercial capillary electrophoresis is a spectrophotometric detector. In the detector, the capillary serves as the detection cell, which simplifies the detector construction, but it also has a substantial drawback. The small inner diameter of the separation capillary results in a short optical path length, leading to reduced sensitivity according to Beer's law. In the case of carbohydrate separation by CE, the simplest solution is the use of UV absorbing probe for the indirect UV detection. However, it is proved, as described below, that carbohydrates are UV detectable without the probe using strongly alkaline electrolyte and careful optimised detection wavelengths. The applicability of the method is evaluated by analysing both food and non-food samples.

8.1.1 In-capillary reactions associated with direct UV detection of neutral carbohydrates

The current study presents a separation method where in-capillary reactions of carbohydrates occur in an alkaline separation medium under the influence of electricity. Those reactions affect the molecules by converting the non-UV-absorbing compound to UV detectable ones (Paper **I**).

The carbohydrate analytes used in the experiments covers a selection of aldohexoses (D-(+)-glucose, D-(+)-mannose, D-(+)-galactose), aldopentoses (D-(-)-arabinose, D-(-)-ribose, D-(+)-xylose), ketoses (D-(-)-fructose), disaccharides (sucrose, D-(+)-cellobiose), sugar alcohols (xylitol, D-(-)-mannitol) and deoxyhexoses (D-(+)-fucose, L-rhamnose).

The alkaline aqueous solution induces ionisation, mutarotation, enolisation and formation of enediolate anions of carbohydrates [1]. The pH of the electrolyte solution used was 12.6, which means that all of the studied carbohydrates were ionised. The ionisation of aldoses occurs on the hydroxyl group of C(1), which has a pKa value of 12.3 [204]. Ketoses are ionised in alkaline solutions via a keto-enol rearrangement.

The detected UV absorbing anion is the carbohydrate enediol, as reported by Petuely and Meixner [205, 206], Kenner and Richards [207], and Ziderman et al. [208].

Enediol anion (Figure 7, Chapter 3.2) is the conjugate base of a carbonyl compound and is resonance stabilized. Ketoses equilibrate to aldoses in alkaline solution via mutarotation. The alkaline ionisation products of carbohydrates possess conjugated enol carbonyl that generates UV absorption at 265 nm – 278 nm [205, 206, 209]. Presumably, sodium cation present in the electrolyte solution is able to interact with the formed anion, preventing the reaction from proceeding to carboxylic acid products. A reaction scheme for non-reducing monosaccharides and disaccharides is presented in Figure 19.

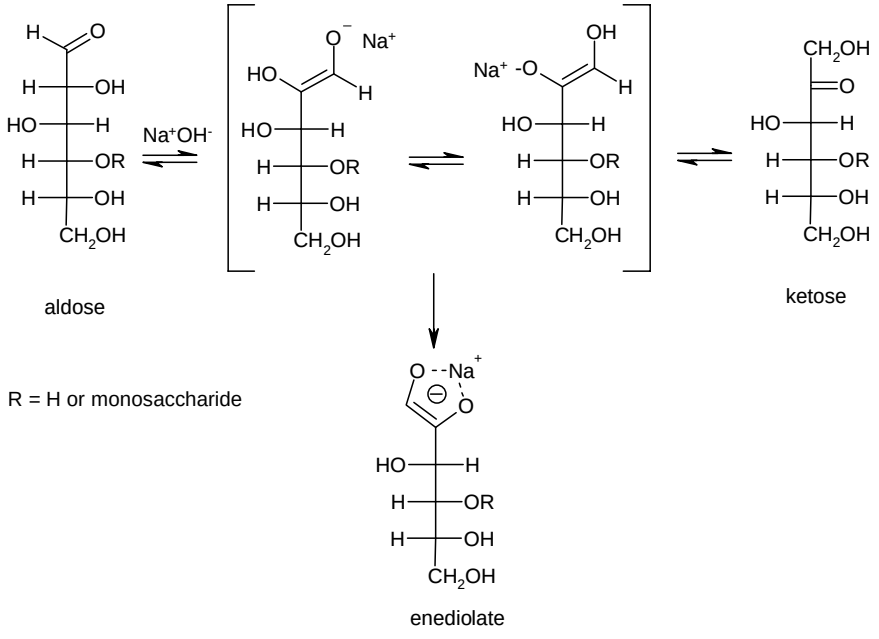

Figure 19. Reaction scheme for the formation of the UV detectable carbohydrate (I).

The other absorption maximum was observed at lower wavelengths, around 185–195 nm. Unfortunately, these signals are disturbed by the overlapped absorption of sodium hydroxide, which can be seen as a wide yellow zone over the whole migration window [210]. Figure 20 shows the contour plot of the separation of some neutral monosaccharides and cellobiose in the range of 190–400 nm and individual peaks spectra. The UV absorption signals of the compounds emerge from the background as yellow – red zones between 250–290 nm. Cellobiose (glucose-β(1→4)-glucose) has distinctly weaker absorption than monosaccharides.

Some generalisations can be made concerning the migration order of the carbohydrates. The first group contains sugar alcohols, which migrate immediately after the electroosmosis. The second group contains disaccharides, and the third group monosaccharides, aldohexoses, ketohexoses, and aldopentoses. Furthermore, 6-deoxy carbohydrates migrate before their parent compounds, *i.e.*, 6-deoxy-D-

galactose [D-(+)-fucose] before galactose and 6-deoxy-L-mannose [L-rhamnose] before mannose.

The reaction mechanism presented here (Paper **I**) let us to assume that epimers, for example glucose and mannose, should produce an identical enediol anion. However, in a strong alkaline medium, the carbohydrates are ionised and rearranged to various degrees, which affects the formation of enediol [35]. As can be seen in Figure 20, the signal intensities of glucose and mannose are slightly different; thus the reaction kinetics varies between epimers. In addition, under highly alkaline conditions, carbohydrates can be separated directly by zone electrophoresis based on the differences between their dissociation constants [101].

Rhamnose, *i.e.* 6-deoxy-L-mannose, produces a noticeably lower signal than its neighbouring analytes glucose and mannose. The methyl group at C(6) may be the reason, but a similar structure is found in the fucose molecule, 6-deoxy-D-galactose, that has a relatively intense signal. Figure 22 shows that the intensity of the signal of rhamnose increases relatively more than that of glucose with increasing NaOH concentration. This may indicate that the reaction condition for L-rhamnose should be even harsher than the 150 mM sodium hydroxide solution offers.

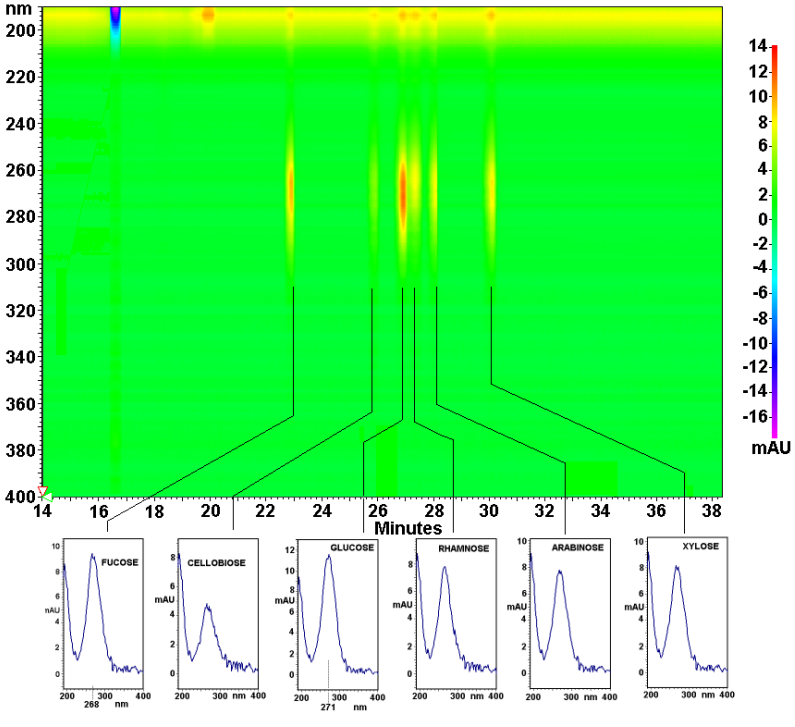

Figure 20. Contour plot of the separation of six carbohydrates, 200 mg L^{-1} in H_2O each, under non-optimised conditions. Below contour plot is depicted UV spectrum of each analyte. BGE solution: 75 mM Na_2HPO_4, pH 12.4, separation conditions: capillary 70/80 cm (L_{det}/L_{tot}), i.d. 50 µm, +25 kV, temperature 25 °C, injection 0.5 psi 30 s [unpublished result].

Another explanation for the direct UV detection mechanism is recently proposed by Sarazin et al. [211]. They reported that instrumental setup has a crucial effect on the direct UV detection of carbohydrates, disaccharides and sugar alcohols. In Beckman Coulter's CE instrument equipped with diode array detector, the light from a deuterium lamp with a continuum of 160–400 nm emission does not go past a filter before reaching the detection window burned in capillary. This means that the sample is irradiated by UV light, which initiates a photochemical reaction.

According to the studies of Laurent [212, 213] and Phillips and Moody [214] irradiation of carbohydrates by UV light generates a strong absorption band which has a maximum at 265 nm. It was noted that the most active wavelengths were below 280 nm [213]. In addition, results show that the intensity of the absorption was increased with the increase of the alkalinity [212] or heat [212].

Bucknall et al. [215] used pulse radiolysis and γ-radiolysis to elucidate the reaction mechanism of the degradation of carbohydrates under radiation and the formation of UV absorbing species. In the proposed reaction mechanism a hydroxyl radical ($HO^{\bullet}$) has a crucial role in the degradation of carbohydrate molecule. In short, the degradation is initiated by the reaction of $HO^{\bullet}$ and C(5) or C(6) of carbohydrate which is followed by ring opening reaction and alkali-catalyzed water elimination from carbohydrate, and split off producing malonaldehyde and dihydroxyacetone (Figure 21). In the case of disaccharides, possible reaction sites are C(5') and C(6') for nonreducing sugars such as sucrose and C(5) for reducing sugar like maltose and lactose.

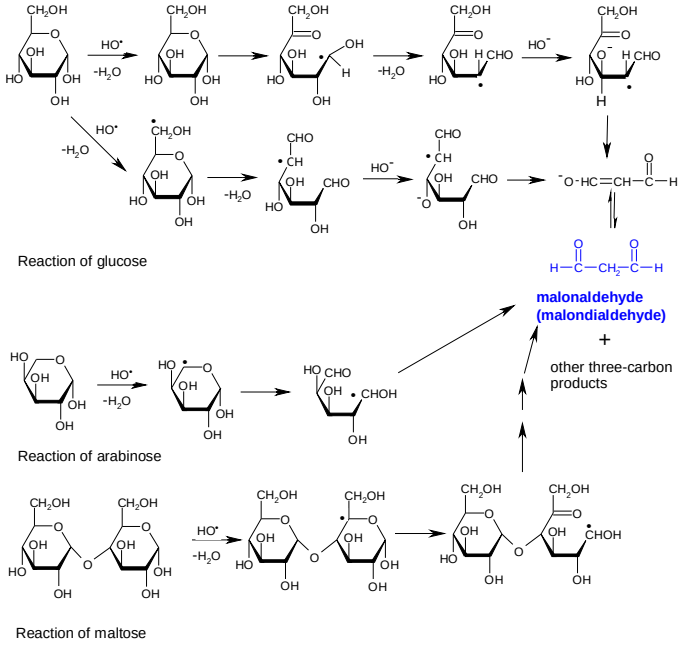

Figure 21. Reaction of hydroxyl radical ($HO^{\bullet}$) at C(5) (upper route) and C(6) (lower route) in glucose. Some hydrogen atoms are leaved out for clarity. Modified from ref. [215].

8.1.2 Effect of the alkali type on the separation

Different alkali chamicals were studied during the optimisation process of separation for analysing monosaccharides, disaccharides, sugar alcohols and combinations.

The study showed that sodium hydroxide concentration influenced both signal intensity and resolution between adjacent analytes. In addition, the concentration of alkali affects the separation current as the instrumental parameter and, therefore, it was take in account during the electrolyte optimisation process.

Figure 22 shows the effect of alkali concentration on the resolution and signal intensity. The best sensitivity was achieved with 100 mM concentration, but the optimum resolution among glucose, rhamnose, and arabinose was gained with the NaOH concentration of 120–130 mM. The ionic strength (*I*) varied from 0.19 M of 100 mM NaOH – 36 mM Na_2HPO_4•$2H_2O$ solution to 0.24 M of 150 mM NaOH – 36 mM Na_2HPO_4•$2H_2O$ electrolyte. Increasing ionic strength decreased the mobility and improved the resolution. Figure 22 also presents clearly that a threshold concentration of alkali, above 100 mM, is needed for the chemical reaction and to achieve a quantitative UV signal of carbohydrates.

Potassium hydroxide, instead of sodium hydroxide, was tested as the chemical of the electrolyte solution. Figure 23 presents a comparison of potassium hydroxide (KOH) and sodium hydroxide (NaOH) electrolytes. Carbohydrate separations occurred faster with KOH, but the resolution of the compounds was poorer.

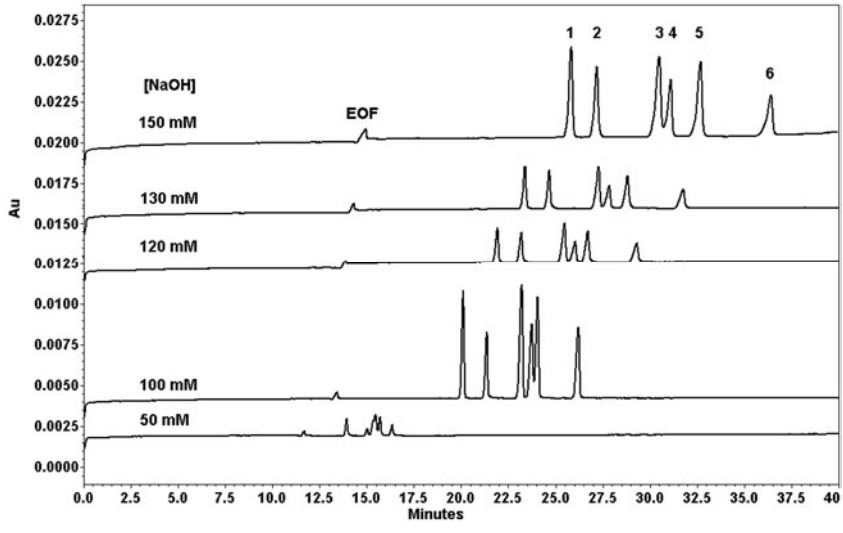

Figure 22. Effect of sodium hydroxide concentration on the separation of six carbohydrates. Peak identities: 1 FUC (164 mgL^{-1}), 2 CEL (342 mgL^{-1}), 3 GLU (180 mgL^{-1}), 4 RHA (164 mgL^{-1}), 5 ARA (150 mgL^{-1}), 6 XYL (150 mg L^{-1}). Sodium phosphate (Na_2HPO_4 •$2H_2O$) concentration in all electrolytes was 36 mM. Separation conditions: +20 kV; detection: 270 nm direct mode; injection pressure, 0.5 psi for 10 s; capillary, 70/80 cm (L_{det}/L_{tot}); separation temperature, 25°C. (Paper I)

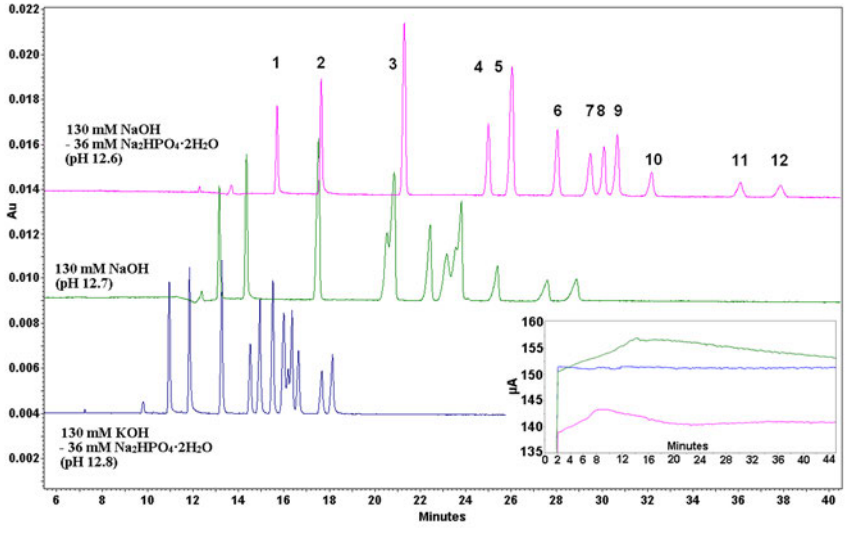

Figure 23. Comparison of NaOH and KOH based electrolytes in the analysis of 12 carbohydrates. Peak identities: 1 xylitol (152 mgL^{-1}), 2 mannitol (182 mgL^{-1}), 3 SUC (342 mgL^{-1}), 4 FUC (164 mgL^{-1}), 5 CEL (324 mgL^{-1}), 6 GAL (180 mgL^{-1}), 7 GLU (180 mgL^{-1}), 8 RHA (164 mgL^{-1}), 9 MAN (180 mgL^{-1}), 10 ARA (150 mgL^{-1}), 11 XYL (150 mgL^{-1}), 12 RIB (150 mgL^{-1}). Separation conditions: +16 kV, detection 270 nm direct mode, injection pressure 0.5 psi for 4 seconds, capillary 50/60 cm (L_{det}/L_{tot}), separation temperature 15 °C. The inset shows the separation current of each electrolyte during separation (Paper I).

8.1.3 Effect of phosphate on the separation

The addition of disodium hydrogen phosphate to the alkaline electrolyte solution resulted in an improvement in resolution between closely migrating hexoses, when compared to pure alkaline electrolyte.The resolution improved remarkably, but as noted earlier (Figure 22) the migration of the analytes was slower. In addition, the separation current was lower, which give more freedom to increase the separation voltage, when necessary.

Some experiments were done with tribasic phosphate, Na_3PO_4. The phosphate anion PO_4^{3-} has a pKa value of 12.38, while the pKa value of hydrogen phosphate HPO_4^{2-} is 7.2 [6]. According to the pKa value of the phosphate anion, the aqueous solution of phosphate has sufficient alkalinity to ionise most of the monosaccharides. However, it was noticed that hydroxyl anions were necessary to generate enediol anions.

8.1.4 Technical aspects concerning the repeatability of the separation

The optimised resolution and migration speed was achieved with the capillary length of 60 cm. It was noticed that the increased analyte migration time in the longer separation capillary was poorly compensated by either by increasing voltage or by a temperature increase from 15 °C to 20 °C. The reason was lower resolution, increased current and increased heat dissipation. On the other hand, when the capillary was shorter than 60 cm, resolution between compounds was diminished, especially between closely migrating glucose, rhamnose, and mannose.

The other technical optimisation made, to improve repeatability of separation, was targeted at the rinsing cycle performed between analyses. It was noted that carbohydrates and their anions have a tendency to adsorb onto the capillary wall. If only one rinsing step with the electrolyte was applied, then the resolution between mannose and arabinose was diminished and the current was decreased by a value of more than 10 µA in consecutive analyses. A rinse with 10% (v/v) acetic acid before the electrolyte washing helped to stabilise the current and the resolution between mannose and arabinose. In addition, a positive effect on repeatability was also obtained by increasing from 0.17 minutes to 1–2 minutes the time taken to raise the separation voltage from zero to the required voltage.

Buffer depletion is a process that often limits the number of runs that can be performed using a set of buffers. In this study, strong buffer depletion was observed when alkali concentrations of 75–100 mM were used. An example of the phenomenon is presented in Figure 24. As anions migrate from the catholyte solution, the electroneutrality is maintained through electrolysis at the surface of the electrode, producing hydroxide ions. This process raises the pH in the catholyte. The corresponding process occurs in the anolyte solution, causing the pH to decrease because of the production of protons via electrolysis [216]. The buffer depletion causes a decrease in both peak area and height in the electropherograms from the first two successive runs. However, both stabilise to a certain level after three or more analyses. In fact, the phenomenon has no effect on the electrophoretic mobilities and changes in both peak area and height can be compensated for by using an internal standard in the quantification. In addition, in order to ensure good repeatability, fresh electrolyte, both in inlet and outlet vials, was used for each analysis. After these improvements, the performance of a capillary was excellent during a 3-week period.

Decreasing the capillary internal diameter from 50 µm to 25 µm was not favourable in respect of detection sensitivity. However, benefits of the smaller capillary are faster analysis, better resolution and, to some extent, better repeatability of the analysis due the smaller depletion phenomenon (Figure 25).

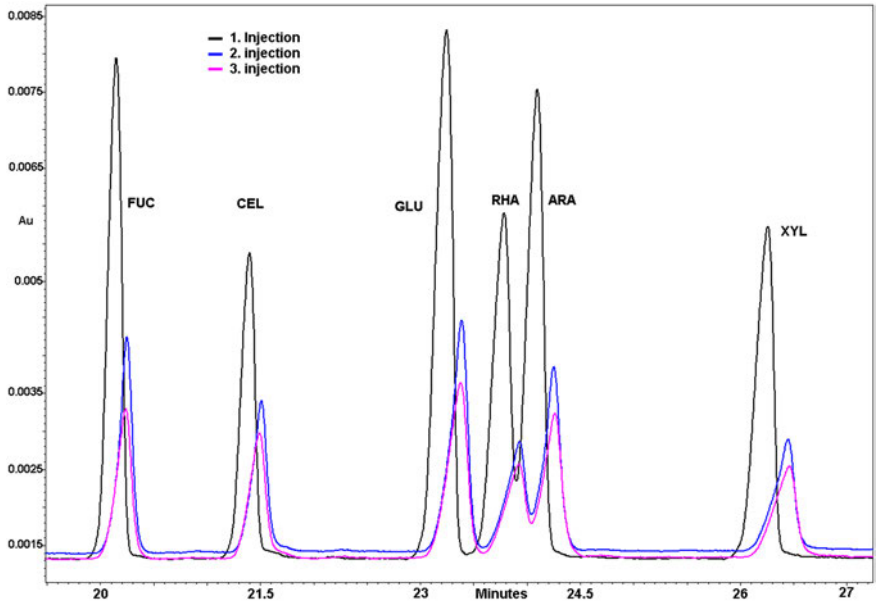

Figure 24. The buffer depletion phenomenon of the separation in three consecutive injections. Concentration of monosaccharides was 200 mg L^{-1} each. Separation conditions: +20 kV, detection 270 nm direct mode, injection pressure 0.5 psi for 20 seconds, capillary 70/80 cm ($L_{det}/L_{tot.}$) 50 µm i.d., separation temperature 20 °C. Electrolyte: 100 mM NaOH - 36 mM $Na_2HPO_4 \cdot 2H_2O$.

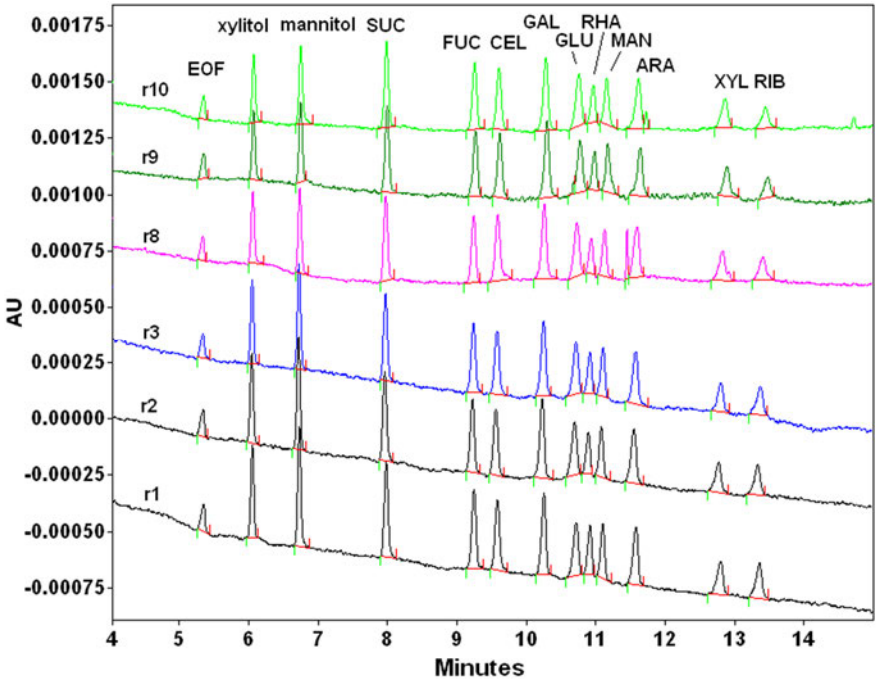

Figure 25. The repeatability using 25 µm (i.d.) capillary in the separation of carbohydrates in ten consecutive injections. Concentration of monosaccharides was 100 mg L^{-1} each. Separation conditions: +15 kV, detection 270 nm direct mode, injection pressure 0.5 psi for 10 seconds, capillary 30/40 cm (L_{det}/L_{tot}), separation temperature 20 °C. A voltage of -27 kV was applied for 5 minutes after migration of the last analyte [S. Rovio 2011, unpublished].

8.2 Method development for carboxylic acids

The determination and identification of water-soluble organic acids can be used to explain reaction mechanisms in various chemical processes involving lignocellulosic materials, since acids are recognised as degradation products of both carbohydrates and lignin. A simple and reliable method for the fast determination both low molecular weight carboxylic acids and selected inorganic anions was developed.

Two modifications of the background electrolyte were developed (Papers **III** and **V**). The first, presented in Paper **III** was fast and suitable for the analysis of the main acids of both alkaline and acidic samples. The second (Paper **V**) was chemically modified from the first electrolyte for the analysis of hydroxy acids in lignocellulosic material. The chemistry of the BGE solution, *i.e.* in chemical composition, pH, ionic strength, and modifiers, influences the resolution of analytes, and their separation efficiency. The goal of method development was to generate a robust BGE solution and a repeatable separation for a large selection of struc-

turally closely related low molecular aliphatic carboxylic acids found in process solutions of lignocellulosic materials.

The aliphatic carboxylic acids do not absorb UV light, therefore 20 mM 2,3-pyrazinedicarboxylic acid (2,3-PyDC) was added, as the chromophore, to the BGE solution for monitoring the acids by indirect UV detection. The detection was performed at 281 nm, where the absorbance of the chromophore was the highest. In addition, the effective mobility of 2,3-PyDC was similar to the organic aliphatic acids under analysis conditions, which ensured good sensitivity and near symmetrical peak profiles.

The best-suited pH value of the electrolyte solution, with respect to resolution and total analysis time, was found to be 8.06±0.02, where all the compounds and chromophore were fully ionised; but an even wider pH range, 8–8.5, was useful without a significant effect on resolution. The pH of the organic buffer solution was adjusted, either with sodium hydroxide (Paper **III**) or with triethanolamine (TEA) (Paper **V**). Sodium hydroxide is a common base for pH adjustment, but here, accurate pH control was challenging due the sudden pH change above pH 5, which is slightly more than the dissociation constant of 2,3-PyDC (pKa 4.12). Therefore, triethanolamine was the next choice for pH-controlling compound. TEA provided pH buffering, but without the loss of sensitivity. Its buffering range is from pH 7.3 to 8.3 [152]. However, buffering capacity was still limited to the concentration of the 2,3-PyDC, since the TEA was added near in equal concentration to that of the chromophore anion.

An organic buffer was chosen to compensate for pH changes and to reduce baseline noise. The zwitterionic tricine (*N*-[tris(hydroxymethyl)methyl] glycine, pKa 8.15) was selected, since its useful working pH range was 7.4–8.8. In Paper **III,** its concentration in the BGE was 65 mM while Paper **V** presents a BGE solution where the concentration was reduced to 30 mM. Buffering of the electrolyte with tricine and adjusting the pH with TEA decreases the separation current and thus reduces the Joule heating generated in the capillary during separation.

The lower ionic strength obtained by the optimisation of concentration of organic chemical and pH adjustment with organic amine allowed a widening of the repertoire of analytes toward hydroxy acids, which migrated after acetic acid.

Cetyl trimethylammonium bromide (CTAB) as a dynamic capillary coating reagent was added to the electrolyte solution in order to reverse the direction of the electroosmotic flow (EOF) towards the positive detector end. TEA acted also as a cell wall modifier [152] and these two components together may prevent the formation of adsorption in capillary wall.

Cations of the alkali earth group have strong tendencies to form partially dissociated metal complexes in solution with the anions of carboxylic acids and other protonated weak acids. Barium cation was used for the complex formation, and therefore barium chloride was added to the electrolyte solution at a concentration of 2 mM. Barium was selected as the metal since it had a positive effect, especially on the resolution of acetate and glycolate, which are unresolved from each other without barium. Moreover, the presence of the bivalent metal cation in the electrolyte solution improves the selectivity and resolution between all dicarboxylic acids

with a backbone of four carbon such as fumaric, maleic, succinic, malic and tartaric acids. Another finding was that Ba^{2+} interacts with carbonate. Therefore, under the experimental conditions, it was supposed that barium forms chelates with carbonates, which have low solubility and may produce dymanic coating on to the capillary surface and thereby affect the repeatability of analysis.

Traditionally, urea has been used as a denaturant in the field of proteomics [217]. Addition of 2 M urea in the BGE increased the viscosity of the solution and resulted in an improvement of the separation between maleate, succinate and malate, thus, between four-carbon dicarboxylic acids. In addition, urea forms hydrogen bonds with carboxylic acids. The oxygen of hydroxyl of the carboxylic group acts as an acceptor in a hydrogen bond and the nitrogen of urea acts as a donor [218].

In summary, the two electrolyte compositions described above produced a chemical matrix which, together with optimised instrumental parameters, produced fast and reliable separation methods for the analysis of aliphatic carboxylic acids.

Monocarboxylic acids migrate according to the number of carbons in the main carbon chain (Figure 26). In general, hydroxy acids follow a similar trend. However, hydroxy acids malic acid and tartaric acid, which have four carbons in their carbon chain, migrated in region of of dicarboxylic acids (Figure 27). The additives of the BGE, especially Ba^{2+} and urea, affect the relative migration order of the dicarboxylic acids having four carbons in their chain. The chemical composition of the BGE solution (Paper **V**) is also good for the separation of aromatic carboxylic acids, but also for the determination of this kind of acids (Figure 26).

Although an indirect detection is applied, the detection sensitivity is quite low. The injected sample volume is 4 nL and 8.5 nL, which means that the injected sample amount is as low as 0.2–0.4 ng when the instrumental conditions presented in Figures 26 and 27 is applied to a sample concentration of 50 mg L^{-1}.

Peak recognition of unknown signals is based on standard addition; a signal remains unidentified without a proper reference compound. However, due to the closely migrating, or even overlapping, peaks, especially in the time window of detection after glycolic acid, false positive identification may occur, as can be concluded from Figure 26 and 27. Therefore, peak recognition with a known reference compound is obligatory.

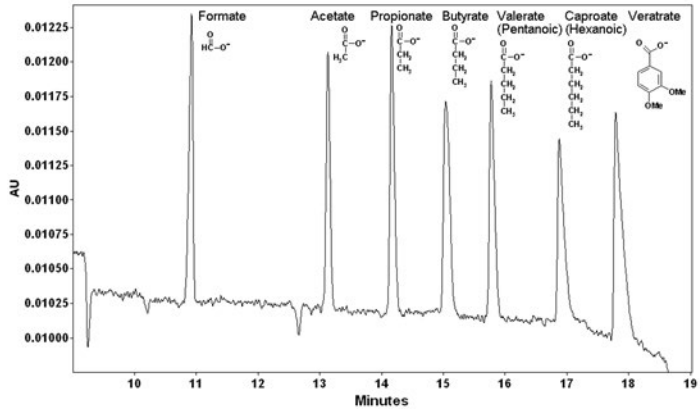

Figure 26. Migration order of selected anions of monocarboxylic acid (50 mgL^{-1} each) and one aromatic acid (100 mgL^{-1}). Electrolyte from Paper V. Separation conditions: capillary (L_{det}/L_{tot}) 50/60 cm, 50 µm i.d., injection 0.5 psi 5 s, separation voltage -8 kV, temperature 15°C, detection 281 nm, indirect detection mode [S. Rovio, unpublished data, 2011].

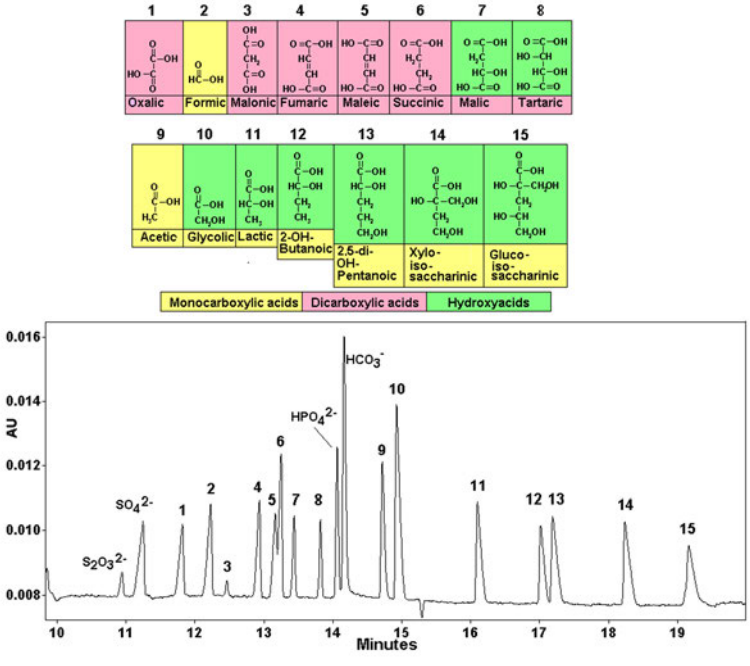

Figure 27. Migration order of selected anions of carboxylic acids and inorganic anions. Electrolyte from Paper V. Separation conditions: capillary (L_{det}/L_{tot}) 100/110 cm, 50 µm i.d., injection 0.5 psi 20 s, separation voltage -25 kV, temperature 15°C, detection 281 nm indirect detection mode. Colour codes depict the nature of acid [S. Rovio, unpublished data, 2011].

The developed CE method was compared with the well-established GC/MS method [188]. The alkali oxidised (oxidation conditions: 0.25 M Na_2CO_3 120 °C for 4 hours, Paper **V**) spruce sample was analysed using both methods. Prior to CE analyses, the sample was diluted with 20 mM NaOH to ensure full ionisation and to open the lactone structure of glucoisosaccharinic acid. For the GC/MS analysis, the sample was derivatized with per(trimethylsilyl) reagent. Both methods characterised the same acids (Table 13), but the percentage proportions of total identified compounds varied. The differences derived from the separation efficiencies of the methods and detection sensitivities of the monitoring systems. The “roughness” of the CE method can be seen in the monitoring of malonic acid and glucoisosaccharinic acid, because the UV detector recorded only one peak per acid and the mass spectrometer also identified, more accurately, the isomeric forms of acids based on the mass spectra library comparison. Furthermore, a large number of other carboxylic acids were also identified by GC/MS, which were not recognised by CE due to the lack of pure standard compounds. However, an advantage of CE method over the GC/MS is the ability to separate and determine volatile acids such as formic and acetic acid together with non-volatile acids. In addition, the careful optimisation of the separation conditions in CE allows the analyses of target compounds with high selectivity, even from very complex sample matrices. The benefit of mass spectrometric detection in GC is the ability to generate and utilise a spectral library for peak identification. Comparison of these results with the data listed in Table 3 reveals that, in our experiments, similar acid compositions were found here to those identified in other oxidation experiments.

Table 13. Comparison of the Developed CE method and GC/MS method in the analysis of oxidation sample of spruce chips [S. Rovio and Klaus Niemelä, unpublished data, 2011].

Compound identified with CE	% of total identified compouds		Compound identified with GC/MS	Other compounds found by GC/MS
	CE	GC/MS		
3-Hydroxypropanoic	0.1	2.7	3-Hydroxypropanoic	1,1,2-Ethanetricarboxylic
Maleic	0.3	2.2	Maleic	Hydroxy-methoxybenzenedicarboxylic
Malonic	0.7	1.1	Malonic	2-OH-Glutaric
		0.4	Malonic (enol)	Glycerol
Malic	0.8	1.4	Malic	Glyceric
2,5-Dihydroxypentanoic	0.9	2.0	2,5-Dihydroxypentanoic	Tartronic
GISA (Glucoisosaccharinic acid)	1.4	0.4	alpha-GISA	Methyltartronic
		0.3	alpha-GISA (1,4-lactone)	Threonic
		0.2	beta-GISA (1,4-lactone)	Erythronic
		1.6	beta-GISA	4-OH-butanoic
2-Hydroxybutanoic	1.8	0.4	2-Hydroxybutanoic	2,4-Dihydroxybutanoic
Succinic	2.2	1.9	Succinic	3,4-Dihydroxybutanoic
Fumaric	2.3	0.9	Fumaric	3-Deoxy-erythro-pentaric
Lactic	5.1	6	Lactic	3-Deoxy-threo-pentaric
Oxalic	8.5	17.3	Oxalic	Pentonic acid
Glycolic	17.5	26.2	Glycolic	3-Deoxy-erythro-pentonic
Acetic	27.8	--		3-Deoxy-erythro-pentonic (1,4-lactone)
Formic	30.6	--		3-Deoxy-threo-pentonic (1,4-lactone)
				3-Deoxy-threo-pentonic
				3-Deoxy-hexonic acid

8.3 Capillary electrophoresis in characterisation of lignocellulosic process samples

8.3.1 Carbohydrates in lignocellulosic samples

Carbohydrate composition originating from polysaccharides in liquors from solid lignocellulosic materials can be determined after their hydrolysis to neutral monosaccharides with mineral acids. Two-stage acid hydrolysis is a standardised pretreatment method [219]. The quantitative results of neutral carbohydrates analysed using CZE are compared with those obtained by HPAEC-PAD (Paper II). L-rhamnose was not detected by CZE whereas it was observed by HPAEC-PAD. Most probably, its signal overlaps with that of glucose due the high concentration difference between these adjacent compounds. As the HPAEC results show (Table 14), the concentration of rhamnose is rather low and the detection limit in CZE is quite high for all carbohydrates studied, 5 mg L^{-1}. The results obtained by CZE and HPAEC-PAD are comparable in terms of quantification, but the RSD values of the peak areas are higher in CZE. Moreover, the total amount of carbohydrates was generally lower in the CZE samples than in corresponding the HPAEC ones. Detailed presentation of quantitation results is reported in Paper **II**.

Table 14. Neutral carbohydrates determined in five fiber samples after acid hydrolysis by capillary elecetrophoresis and HPAEC (Paper II).

Sample		mg /100 mg dry weight (RSD %)							
		L-Rhamnose	D-(-)-Arabinose	D-(+)-Galactose	D-(+)-Glucose	D-(+)-Xylose	D-(+)-Mannose	Total amount of mono-saccharides	Dry weight %
Oat spelt	CZE	n.d.	1.9 (20)	0.8 (19)	27 (13)	25 (7.4)	n.d.	55.6	94.7
	HPAEC	*< 0.1*	*2.8 (3)*	*1.3 (3)*	*32 (1)*	*30 (3)*	*<0.5*	*66.7*	
Wheat straw	CZE	n.d.	1.7 (30)	0.5 (11)	40 (4.3)	16 (7.9)	0.2 (19)	59	94.9
	HPAEC	*< 0.1*	*2.2 (9)*	*0.8 (8)*	*42 (8)*	*23 (9)*	*0.4 (10)*	*69.1*	
Spruce TMP	CZE	n.d.	n.d.	2.0 (2.3)	49 (13)	5.5 (4.8)	14 (8.1)	70.6	94.2
	HPAEC	*0.14 (7)*	*1.2 (2)*	*1.9 (3)*	*49 (4)*	*5.6 (3)*	*13 (2)*	*71.9*	
Aspen stemwood	CZE	n.d.	1.1 (22)	0.4 (16)	48 (12)	18 (3.7)	1.9 (16)	68.8	94.7
	HPAEC	*0.29 (3)*	*0.3 (3)*	*0.5 (2)*	*51 (1)*	*18 (1)*	*2.2 (3)*	*73.9*	
Bleached birch kraft pulp	CZE	n.d.	1.0 (28)	n.d.	86 (16)	25 (11)	0.6 (11)	112	95.7
	HPAEC	*< 0.1*	*< 0.1*	*< 0.1*	*80 (2)*	*25 (1)*	*0.5 (4)*	*105.7*	

8.3.2 Carboxylic acids in black liquor

In the field of lignocellulosic research, capillary electrophoresis has a significant role in the analyses of low molecular weight degradation products, such as aliphatic carboxylic acids, phenols and various inorganic anions present in reaction mixtures. In order to follow reaction kinetics and to determine the reactive pathways, it is necessary to quantify these degradation products and to detect the structural changes in the polymeric lignin [220, 221, 222].

Black liquor is a potential source of hydroxy acids. In alkaline cooking processes more than 20 different carboxylic acids are formed by decomposition of lignocellulose constituents and, especially, due to the reactions of hemicelluloses [Paper **VI**, 188]. From those, the six major hydroxy acids are glycolic, lactic, 2-hydroxybutanoic, 2,5-dihydroxypentanoic, xyloisosaccharinic and glucoisosaccharinic acids, which, except glycolic acid, are exhibited in racemic mixtures [188].

Isolation of hydroxy acids from Kraft black liquors is a challenging task, due to both the complex composition of black liquor and low concentrations of carboxylic acids. Black liquor contains hundreds of different organic compounds from pulping and inorganic cooking chemicals. In addition, while the hydroxy acids are among the most polar constituents in black liquors, they are not spontaneously separable. Isolation and purification of hydroxy acids from black liquors is typically carried out by combination of acidification to precipitate lignin, and purification of the residue by distillation [13]. Unfortunately, distillation is not an adequate method for purification of acids, since some major acids, such as glycolic, lactic, and 2-hydroxybutanoic, have boiling points close to each other. Therefore, for the recovery of the hydroxy acids, ultrafiltration, electrodialysis, and chromatographic are alternative isolation and fractionation methods. After group fractionation, there is still a need for further isolation and purification of individual compounds of mono- and polyhydroxy acids to get sufficiently pure individual acids for further needs [223, 224, 225].

Capillary electrophoresis was applied in the analysis of carboxylic acids of ultrafiltrated samples. Further it was used in preparative ion exchange chromatographic fractionations of black liquor [Paper **VI**, 225]. The electrolyte presented in Paper **V** was applied in these process control tasks.

Figure 28 shows the fractionation of the major hydroxy acids from other black liquor components. Hydrophilic hydroxy acids were the main targets, but volatile acids formic and acetic acid, which were side products, were found in considerable amounts. Ultrafiltration, with pH adjustment, of the sample and crystallisation for separating inorganic matter from the filtrate were found to be efficient means for the removal of lignin and inorganic compounds, as well as for the enrichment and purification of organic compounds in black liquor (Paper **VI**). The preparative chromatography separates anionic compounds from other black liquor components, and further enhances fractionation of carboxylic acids from one another.

A feedback between analysis result and fine tuning of the instrumental parameters of the fractionation systems is essential for obtaining optimal performance in the isolation of valuable compounds. Therefore, it would be beneficial if the analy-

sis of the effluents could be carried out as on-line measurements. Unfortunately, in the above mentioned case studies this feedback loop was not fulfilled optimally; the fractions were collected and analysed as one batch per experiment.

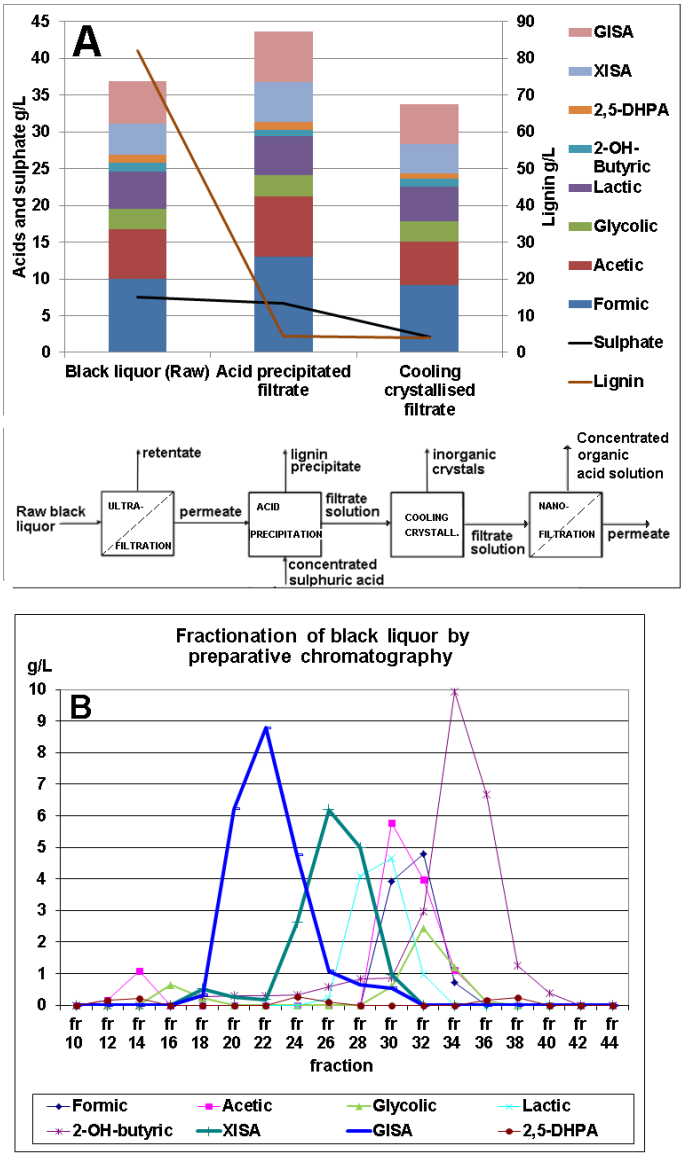

Figure 28. (A). Acid composition, lignin and sulphate content of raw black liquor, after ultrafiltration and acid precipitation, and after crystallisation. Schematic presentation of process flow for separation of carboxylic acids. Modified from Paper VI. (B). Fractionation of carboxylic acids with preparative ion chromatography [225] GISA: glucoisosaccharinic acid, XISA: xyloisosaccharinic acid, 2,5-DHPA: 2,5-dihydroxypentanoic acid.

8.3.3 Alkaline oxidation of wood

Oxidation in alkaline conditions, optionally enhanced with catalyst (Paper **V**), is an effective technique for the fractionation of woody raw materials. It provides a fibre fraction containing readily hydrolysable carbohydrates to be utilized e.g. in biotechnical ethanol production and a liquid fraction containing solubilized lignin and reaction products from various biomass components.

Alkaline oxidation of lignocellulosic material at elevated temperatures and pressures is studied in Papers **V** and **IV**. Reaction conditions, such as temperature of 120–140 °C, oxygen pressure of 1 MPa, reaction time 4–20 hours, and alkali concentration of 0.25 M are employed to fractionate lignocellulosic material into a cellulose-rich fibre fraction and a liquid fraction, which contained dissolved hemicelluloses, lignin and degradation products (Paper **V**).

The carbohydrate content of the spruce raw material was 66–65%. Only 1 to 6% of the carbohydrates in the raw material were found in the soluble fractions. The carbohydrate yield was between 66 and 92% of the raw material carbohydrates. Table 15 shows the distribution of monosaccharides in fibre and liquid fractions. It can be observed that the hemicelluloses, arabinoxylan and galactoglucomannan mainly dissolve, while the cellulose remains in the fiber fraction.

Table 15. Carbohydrate fraction yield and total yield. Yield is expressed as % of the raw material carbohydrate content. The percentage in sample description denotes the filling ratio of the reactor (V).

SAMPLE	Fraction	GLU	MAN	XYL	GAL	ARA	Total yield of monosaccharides (%)
AlkOx 120°C 4 h 75%	solid	103	59	86	47	77	92
	liquid	0.1	1	6	11	14	1
CatOx 120°C 4 h 75%	solid	89	56	61	30	49	78
	liquid	0.7	6	20	21	30	4
AlkOx 140°C 4 h 25%	solid	99	41	71	29	50	83
	liquid	0.2	1	12	14	26	2
CatOx 140°C 4 h 25%	solid	100	48	54	20	38	83
	liquid	1.1	7	28	21	37	6
CatOx 120°C 20 h 10%	solid	106	55	41	6	19	87
	liquid	1.1	14	42	37	52	9
AlkOx NaOH 120°C 20 h 10%	solid	84	35	36	12	24	67
	liquid	1.0	11	31	38	59	8

Figure 29 presents examples of the effect of reaction conditions on the distribution of fiber and liquid fraction as well as proportions of monosaccharides, lignin and carboxylic acids in the liquid fraction.

The portion of dissolved fraction increases when Cu(II)-phenanthroline catalyst was added to the reaction mixture. The catalysed reaction dissolves more wood than its alkaline counterparts does. Lower filling, together with larger head-space volume and increased temperature were also beneficial parameters in enhancing the dissolution. A five-fold increase of the reaction time enhanced the dissolution in the catalysed reaction from 28% to 48%. In addition, CatOx treatment is slightly more effective in dissolution than the AlkOx treatment performed in a stronger alkali (NaOH) medium. In addition, the combination of lower liquid volume and increased oxygen volume probably improves the mixing of suspension, decrease concentration gradients, and thereby enhances the oxidation reaction and solubilisation of hemicelluloses and lignin.

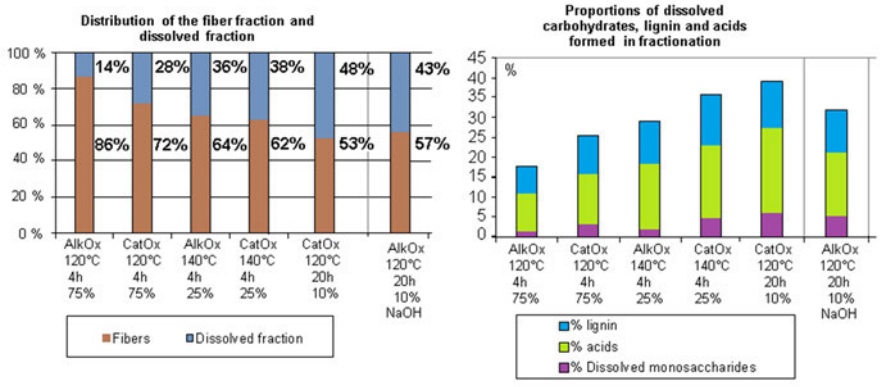

Figure 29. Percentage distribution of the fibre fraction and soluble fraction after oxidation experiments (left). Acronyms: AlkOx = alkaline oxidation, CatOx = catalysed alkaline oxidation. The percentage in sample description denotes the filling ratio of the reactor. Portions of carbohydrates, lignin, and small carboxylic acids found in dissolved fraction are presented in the right Figure. The alkali was 0.25 M Na_2CO_3, unless otherwise stated (Paper V).

Closer examination of the chemical composition of the dissolved fraction revealed that the catalyst in short-duration reactions enhanced degradation and dissolution of hemicellulose, whereas the monosaccharide content was almost at the same level in catalysed and alkali treatments (Figure 29, right). The effect of catalyst on dissolution of lignin is not as obvious as in the case of hemicelluloses. Big variations on the percentage of lignin were not observed, which indicates that the catalyst affected the dissolution of hemicelluloses more than the degradation of lignin.

The generation of carboxylic acids as degradation products of hemicelluloses and lignin follows the dissolution of wood components. When 20-hours reactions times results were compared, the proportion of acids was lower in alkali-oxidised experiments than its catalysed counterpart. Most probably, this phenomenon may be associated with harsher reaction conditions. Sodium hydroxide is a stronger

base than sodium carbonate, and therefore it may provoke the oxidation reactions of carboxylic acids.

The cumulative amount of the recognised dissolved compounds was lower than the dissolved fraction portion. The unexplained part may include other forms of degradation product that cannot be determined with the applied methods and unidentified carboxylic acids. In addition, carbon dioxide may promote alkalisation, since it may be absorbed from air and can be found in such alkaline solution as carbonate. In addition, carbonate is a significant degradation product in the alkaline oxidation of lignin [61].

8.3.4 Alkaline oxidation of lignin

Oxygen delignification is widely utilized in the modern chemical pulp industry. The residual lignin is removed during the subsequent delignification and bleaching stages. Unfortunately, during oxygen delignification, the carbohydrate reactions are increased. Therefore, optimisation of oxygen delignification is needed in order to find the optimum conditions for the removal of residual lignin and to retard polysaccharide degradation. Temperatures of 90 or 110 °C, and pressure, 0.6 or 0.9 MPa, were efficient in breaking down the macrostructure of lignin (Paper **IV**). Oxidation of lignin was monitored as a function of time by means of a number of analysis techniques [226, Paper **IV**] and the obtained information was applied to construct a kinetic model of the oxygen delignification of kraft pulp [61].

During the first 60 min, lignin reactivity was high, but the most intense phase took place during the first 30 min as can be seen in Figure 30. This figure shows, as an example, how the amount of different hydroxylic functional groups, analysed with ^{31}P NMR, developed within four-hours' reaction time. It was noted that, under the studied conditions, an increase in the reaction temperature, rather than the pressure, had primary significance in the degradation rate of lignin. At higher temperatures, the degradation of phenolic OH group is faster, mainly due the degradation of guaiacylic OH units. Similarly, the concurrent formation of carboxylic acids was observed (Figure 30). Thus, alkaline oxidation promotes dissolution of lignin by the formation of muconic acid type structures due the aromatic ring opening – as Asgari and Argyropoulos presented in their study [51]. Simultaneously with partial degradation, lignin became more hydrophilic.

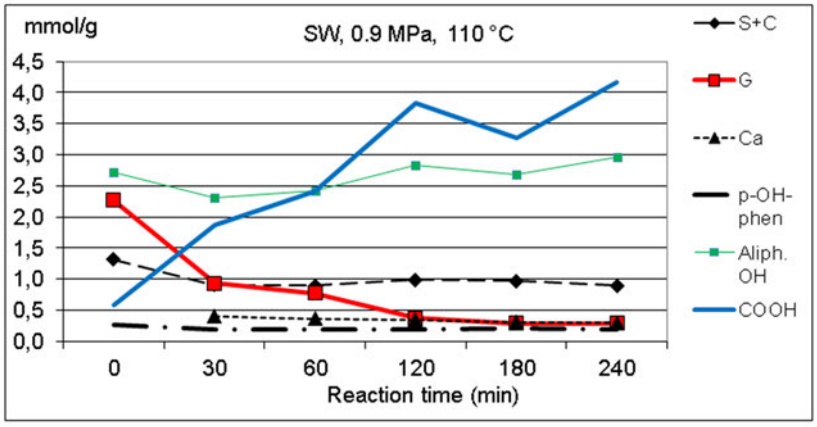

Figure 30. Development of the concentration levels of different hydroxylic functional groups in softwood Kraft lignin under oxidation conditions of 110°C and 0.9 MPa as monitored with ^{31}P NMR spectroscopy (Upper diagram). S+C: phenolic OH in syringylic units and in condensed functional groups, G: phenolic OH in guaiacyl groups, Ca: catechols, *p*-OH-phen: phenolic OH in *p*-Hydroxyphenyl groups, aliph. OH: aliphatic OH groups (alcoholic OH), COOH: various carboxylic acids.

8.3.5 Alkaline oxidation of monosaccharides

Two aldopentoses; arabinose and xylose, and three aldohexoses; glucose, galactose, and mannose were oxidised under mild alkaline conditions. The carbohydrates were chosen according the monosaccharide units found in cellulose and hemicelluloses. The purpose was to evaluate how the monosaccharides are oxidised in similar alkaline oxidation conditions under an oxygen atmosphere that was used in oxidation of softwood. In addition, by comparing the oxidation products of carbohydrates and softwood, it may reveal whether the acid is formed due the oxidation of cellulose or hemicelluloses.

8.3.6 Aliphatic carboxylic acids in alkaline oxidated liquors of spruce wood, softwood lignin and monosaccharides

According to our results, the major organic acids found in both wood and pure lignin oxidated liquors were formic, acetic, glycolic and oxalic acids. The group of minor acids consisted of malonic, fumaric, succinic, malic, and lactic acids, but in the case of spruce wood (**V**), a collection of hydroxy acids, 3-hydroxypropionic, 2-hydroxybutanoic, and 2,5-dihydroxypentanoic acids, was also found, but only in minor concentrations. Figure 31 shows how the oxidation conditions affect the generation of carboxylic acids. In a study on catalysed oxygen delignification of spruce chips, degradation of dissolved monosaccharides and lignin to small aliphatic carboxylic acids was increased in catalytically assisted oxidations com-

pared to alkaline oxidations. The acid profile was similar, both in the presence and on the absence of the catalyst, although slight variations in concentrations of individual acids could be observed. Oxalate, formate and malonate were formed more in catalytic oxidation than in uncatalysed alkaline oxidation. Furthermore, the catalysation produces less succinic and malic acids under 20 hours' treatment than was found in uncatalysed oxidation performed in similar conditions.

The detailed study of lignin degradation revealed that the reactions with end products of carboxylic acids are connected to the ring-opening reactions of phenolates via generation of muconic acid intermediates, disintegration of lignin polymer due the breaking of aliphatic side chains and oxidation reactions of carboxylic acids formed in the previous two reaction cascades. Among the carboxylic acids, formic acid is a primary degradation product in the initial steps of all three reaction routes, while other acids are only formed as degradation proceeds [61].

Our study shows that oxidation of monosaccharides produced only a few aliphatic carboxylic acids, when compared to the acid profile found in process liquors of spruce wood. The four main acids in all experiments were formic, glycolic, glyceric and erythronic acid, covering 70–90% of the total identified acids. Other identified acids were oxalic, acetic, maleic, 3-hydroxypropionic, xylonic and gluconic acids that were found only in trace quantities. Gluconic, xylonic and maleic, eg., carboxylic acids with 4–6 carbons in the carbon chain, were found in hexose samples whereas 3-hydroxypropionic acid was characteristic for pentose sugars.

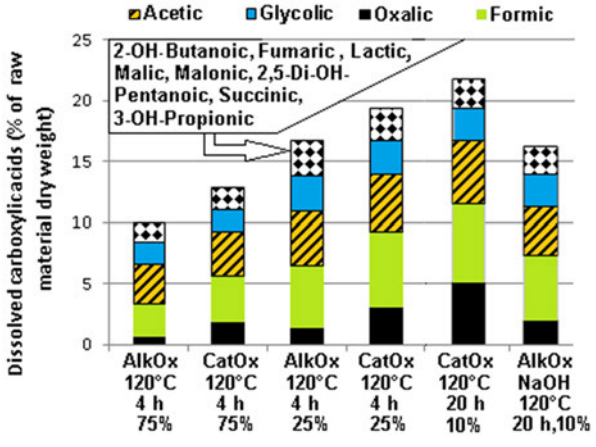

Figure 31. Distribution of four main aliphatic acids and sum of minor acids found in the soluble fraction under different oxidation conditions (V).

Figure 32 compiles the distribution of the major acids produced in the alkaline oxidation of different lignin, softwood and monosaccharide samples. It can be noted that formic acid is a characteristic degradation product of both lignin and monosaccharides. It was found in substantial amounts as a degradation product of wood in general. Acetic and oxalic acids were found in substantial proportions both in

wood and lignin samples, whereas their proportion among monosaccharide oxidation products is clearly lower. Glycolic acid was found as a degradation product in all three groups. Its existence is larger among monosaccharides than among lignin samples. However, wood processing indicates that glycolic acid is formed due to the degradation of oligosaccharides rather than of lignin. The finding correlates with the data in Table 5, where glycolic acid is marked as an oxidation product of all wood polysaccharides. Erythronic and glyceric acids were found only as oxidation products of monosaccharides and their proportions are nearly equal, especially after the oxidation of pentoses. They were not detected in alkaline oxidation liquors of spruce, since erythronic and glyceric acids are the expected structures formed as a result of the stopping reactions in the polysaccharide end groups.

The mutual percentage relations of carboxylic acids varied little inside each group. However, the variations among the spruce samples can be explained with the presence of the catalyst, which enhances the formation of oxalic acid when the comparing CatOx reactions to their AlkOx counterparts in four hours' and 20 hours' reactions. Also, catalysation increases the formation of formic acids. Oxalic acid was not detected in AlkOx experiments of spruce saw dust, where calcium hydroxide was used as alkali. Here oxalic acid was precipitated as calcium oxalate (Figure 32).

The largest variations were found between the oxidations of two pentose monosaccharides, arabinose and xylose, which have clear differences in the proprotions of formic, erythronic and glyceric acids. Moreover, among hexoses, oxidation of mannose produced more oxalic than is found in those of galactose and glucose.

Table 16 compiles the acid composition formed in alkaline oxidation of monosaccharides, lignin, and softwood, as presented here and in Papers **IV** and **V**. In summary, a comparison of the alkaline oxidations of the different raw materials reveals that formic acid is a major degradation product in all three cases. On the contrary, acetic acid is a minor degradation product in the oxidation of monosaccharides, but a major degradation product of lignin and wood. A potential source of acetate is the acetyl groups of glucomannan (Chapter 2.2.2). The functional groups are detached from oligosaccharide backbone by alkali reaction.

The C(3) and C(4) dicarboxylic acids seemed to be typical oxidation products of lignin whereas C(2)-C(6) hydroxy acids produced after oxidation of wood samples refer to the degradation of polysaccharides (Table 5). In addition, these acids have a relevant role as degradation products under alkaline conditions (Table 4). Xylonic and gluconic acids, which are C(5) and C(6) hydroxy acids, can be found only in monosaccharide oxidations, simulating the identified endgroups of oxidised cellulose and xylan [70].

The results concerning lactic acid are consistent with the results of Salmela et al. [191]. This acid is a common oxidation product of polysaccharides obtained under alkaline conditions and it can be found in substantial amounts in alkaline black liquors of both softwoods and hardwoods (Table 3). However, in oxygen delignification liquors, lactic acid has been found in lower amounts. Here it was found to be a minor oxidation product of spruce (**V**, Figure 31, lower). In addition, lactic acid was not detected as an oxidation product of monosaccharides.

According to de Bruijn and his co-workers [227] as well as Yang and Montgomery [44], the degradation of monosaccharides is directed by the reaction conditions.

The degradation of monosaccharides is directed toward lactic acid when the initial monosaccharide concentration is below 0.01 M and/or a hydroxyl anion concentration is above 0.1 M. In addition, the nature of base, particularly divalent cation, Ca^{2+} or Mg^{2+}, favors the formation of lactic acid. The reaction temperature also has an effect on the composition of the reaction products [228].

In our study, the concentration of each monosaccharide (arabinose, galactose, glucose, mannose, and xylose) was appromately 0.3 M, and the divalent cation, Ca^{2+} is replaced with monovalent sodium. Therefore, it can be concluded that our experimental conditions do not promote the formation of lactic acid. In the oxidation of spruce, the formation of lactic acid is clearly higher at 140 °C than at 120 °C (Figure 32, lower). In addition, its proportion is higher when calcium hydroxide was used instead of KOH, NaOH or Na_2CO_3.

Moreover, de Bruijn stated [35] that when molecular oxygen is supplied at high pressure, the reaction is directed toward the fission of C(1)–C(2) bond which generates the formation of formic, D-erythronic, D-glyceric, glycolic and arabinonic acids. As shown in Figure 32 (upper), all of these acids, except arabinonic acid, which was not detected at all, are found in substantial amounts in our alkaline oxidation experiments of pentoses and hexoses.

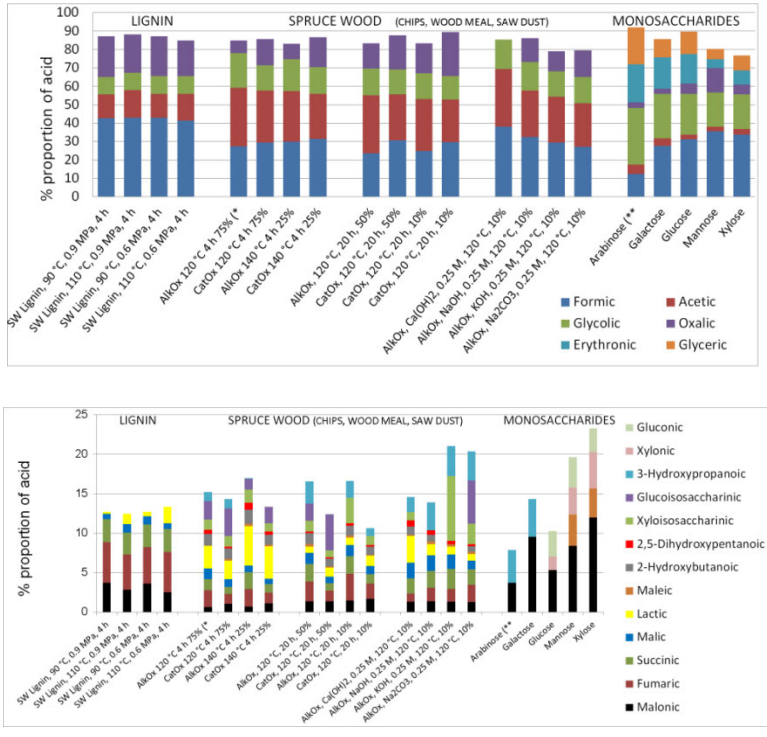

Figure 32. Distribution of the six most abundant aliphatic carboxylic acids (upper) and minor acids (lower) found in reaction mixtures after alkaline oxidation of spruce wood, lignin and monosaccharides. (*filling ratio of reactor, (** 80 °C, 5 h, 2 MPa) (IV, V, unpublished data).

Erythronic and glyceric acids are another pair of interesting oxidation products. They have found in substantial quantities in the alkaline oxidation of monosaccharides, whereas they are absent, or their amount below detection limits, in the oxidation studies of spruce chips, wood meal or saw dust. Erythronic acid is formed from monosaccharide through an oxidative α-dicarbonyl cleavage [45, 76]. However, erythronic acid is a stabilising end group in the stopping reactions of cellulose degradation while glyceric acid is recognized as a stabilizing end group of xylan (Figure 13). Thus, erythronic and glyceric acids are the expected structures formed from the monosaccharides, simulating the structures formed as a result of the stopping reactions in the polysaccharide end groups.

Carboxylic acids found in alkaline oxidation experiments of lignin are similar to those reported by Sun et al. [229]. They used UV-assisted alkaline perodixe oxidation for the oxidation of guaiacyl, syringyl, veratryl and 3,4,5-trimethoxyphenyl lignin model compounds. According to their results, dicarboxylic acids are formed due the ring-opening reactions of aromatic model compounds. Examination of minor oxidation products of spruce reveals that fumaric acid is generated more in AlkOx treatments than in CatOx experiments whereas the catalysation favours the generation of malonic acid, especially when reaction time is four hours.

Table 16. Compilation of carboxylic acid compositions found in the alkaline oxidations of pentoses and hexoses, lignin, and softwood. Bold font indicates hydroxy acids and italic font dicarboxylic acids. Symbols: ●Major acid, ○ minor acid. C1-C6: Carbons in carbon chain (IV, V, unpublished data).

Carboxylic acid	**Monosaccharides**		**Lignin (SW, HW)**	**Wood (SW)**
	Pentoses	Hexoses		
Reaction conditions Alkali: Reaction time: Oxygen pressure: Temperature:	Monosaccharide concentration 0.3 M 1.0 M Na_2CO_3 pH 9.0 5 h 2 MPa 80 °C		 1.0 M NaOH 4 h 0.6 / 0.9 MPa 90 / 110 °C	Liquid – wood ratio ~20 0.25 M Na_2CO_3, NaOH, KOH, $Ca(OH)_2$ 4 h / 20 h 1 MPa 120 / 140 °C
C1				
Formic	●	●	●	●
C2				
Acetic	○	○	●	●
Glycolic	●	●	●	●
Oxalic	○	○	●	●
C3				
3-Hydroxypropionic	○	○		○
Glyceric	●	●		
Lactic			○	○
Malonic			○	○
C4				
2-Hydroxybutanoic				○
Erythronic	●	●		
Fumaric			○	○
Malic			○	○
Maleic	○	○	○	○
Succinic			○	○
C5				
Xylonic	○	○		
2.5-Dihydroxypentanoic				○
Xyloisosaccharinic acid (xisa)				○
C6				
Glucoisosaccharinic acid (gisa)				○
Gluconic	○	○		

9. Concluding remarks

The current study presents an alternative capillary electrophoretic separation method to CE methods based on indirect UV detection or on precolumn derivatisation for the analysis of mono-, di-, and oligosaccharides and sugar alcohols. Strong alkaline electrolytes can be considered an exceptional choice for the separation medium in respect to ionic strength and pH, since, in general, the combination of those two attributes should be kept at a moderate level in order to avoid Joule heating and dispersion effects. This study shows that, due to the high alkaline concentration and applied electrical field, the in-capillary reaction for direct detection of carbohydrates becomes possible. However, further optimisation is needed to reduce analysis time from the current 30 minutes to well below 20 minutes in order to enhance sample throughput times. In addition, improvements in repeatability are necessary to obtain a reliable analysis method. These two requirements can be fulfilled using 25 µm capillaries, but at the cost of detection sensitivity. The presented CZE method can be applied, for example, to control quality in the food industry. Pulp and paper industry can exploit this method in determination of the composition and concentration of carbohydrates in cellulosic fibres of different plant materials.

A simple, fast, and repeatable method was developed for the analysis of low molecular weight carboxylic acids. The developed separation method showed significant separation efficiency of compounds, mostly due the complex forming cation, which enhances the selectivity of structurally closely related acids. The background electrolyte was proven to be immune to pH variations, high ionic concentrations and matrix compounds found in complex samples. Thus, the application range covers both acidic and alkaline samples. As in the case of carbohydrate analysis, minimal sample preparation is needed prior to analyses.

The CE analyses provided information about the acidic degradation products formed in alkaline oxidation of lignin and spruce wood material. The data obtained with carboxylic acids acid analyses was useful in evaluation of the pretreatment conditions of spruce raw materials. By analysing the dissolved components, it was observed that catalytically assisted oxidation was more efficient than its alkaline counterpart in the dissolution of carbohydrates, especially of those originating from glucomannan and arabinoxylan. The catalyst increased both the degradation and dissolution of hemicelluloses and the degradation of lignin to small aliphatic car-

boxylic acids. The acid profile was quite similar in the presence and absence of the catalyst although some variations in the concentration of individual acids were observed. Comparison of four different bases showed that NaOH, KOH, and Na_2CO_3 were comparable in dissolution efficiencies. Thus, total concentration of acids (analysed e.g. by titration) is not an adequate measure for the evaluation of the reaction conditions.

The composition of major acids obtained both in alkaline oxidation of lignin and spruce wood is similar, and therefore it can not deduce the orginal source of the oxidation product. However, it can be noted, that among the minor acids dicarboxylic acids, malonic, fumaric, and succinic acids, are the marker for the lignin degradation and the existence of hydroxy acids in process liquors indicates the degradation and oxidation of hemicelluloses. The presence of glyceric, erythronic, xylonic and gluconic acids in process liquors reflects the oxidation of monosaccharides, since in alkaline oxidation conditions of hemicelluloses, they are formed in stopping reactions, and attached to hemicellulose as stabilising end groups.

The CE method developed is suitable for following the kinetics of the enrichment process. Mmebrane filtration separates aliphatic carboxylic acids from lignin and inorganic sulphate. The combination of ultrafiltration and cooling crystallisation is an efficient method for recovering and purifying valuable organic compounds from black liquor. Also, the CE method gives valuable information on the effectiveness of preparative chromatographic in the separation of aliphatic carboxylic acid from each other.

In addition, to our knowledge, this is the first time that C(5)–C(6) aliphatic carboxylic acids, i.e. 2,5-dihydroxypentanoic acid, glucoisosaccharinic acid and xyloisosaccharinic acid, have been determined by capillary electrophoresis from black liquor solutions.

In summary, both capillary electrophoretic methods presented have the potential to be applied for demanding bio-related samples. Future prospects cover the widening of the applications of acid method towards the other biorefinery processes, such as organosolv cooking and acidic bioprocesses.

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PAPER I

Determination of neutral carbohydrates by CZE with direct UV detection

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Research Article

Determination of neutral carbohydrates by CZE with direct UV detection

A new CZE method relying on in-capillary reaction and direct UV detection at the wavelength 270 nm is presented for the simultaneous separation of the neutral carbohydrates xylitol, D-(−)-mannitol, sucrose, D-(+)-fucose, D-(+)-cellobiose, D-(+)-galactose, D-(+)-glucose, L-rhamnose, D-(+)-mannose, D-(−)-arabinose, D-(+)-xylose, and D-(−)-ribose. The alkaline electrolyte solution was prepared of 130 mM sodium hydroxide and 36 mM disodium hydrogen phosphate dihydrate. Separation of the sample mixture was achieved within 35 min. Calibration plots were linear in the range of 0.05–3 mM. Reproducibility of migration times was between 0.3 and 1.1%, and the detection limits for the analytes were 0.02 and 0.05 mM. The optimized method was applied for the determination of neutral monosaccharides in lemon, pineapple, and orange juices and a cognac sample. The methodology is fast since no other sample preparation except dilution is required.

Keywords:
CE / Direct UV detection / Neutral carbohydrate DOI 10.1002/elps.200600783

1 Introduction

Carbohydrates are ubiquitous in the living world. In plants and animals they act as energy and carbon sources and they are essential for metabolism. They are important constituents in plant cell walls as well as in the extracellular matrix of animal and human tissues. Carbohydrates such as sucrose, glucose, and fructose are key biological substances in many life processes. They are also widely used as food additives. For product safety and consumer protection, many regulations are in place in regard the amounts of carbohydrates permissible in foods [1].

Determination of carbohydrates in samples of different type and origin is a challenging task in the biological and food sciences [2]. Although a number of analytical techniques and methods have been proposed, most of these are time-consuming due to the complex sample cleanup and derivatization procedures required. The analysis of carbohydrates without derivatization is particularly challenging. Furthermore, carbohydrates can be detected with universal detectors such as spectrophotometers with only low sensitivity, since they absorb UV light within only a limited range (190–210 nm). Fluorescent properties are necessary for direct native fluorescence detection.

Lately, gas and liquid chromatographic techniques combined with mass spectrometric detection have been contributing to carbohydrate research. GC coupled with MS (GC-MS) has frequently been used for the analysis of monosaccharides [3]. The laborious sample preparation required before the analysis is a drawback. Medeiros *et al.* [4] used dichloromethane–methanol mixture to extract mono- and disaccharides from plant matrices and trimethylsilyl (TMS) derivatization before GC-MS analysis. GC has been shown suitable for the determination of mono- and disaccharides in honey after sample cleanup. D-(+)-Glucose, D-(+)-galactose, D-(−)-fructose, D-(+)-maltose, lactose, and sucrose were separated after isolation from honey samples and derivatization with *N*-(trimethylsilyl)imidazole in pyridine [5].

In recent years, there has been notable development in liquid chromatographic techniques for carbohydrate analyses as a result of the coupling of high performance anion-exchange chromatography with pulsed-amperometric detection. This is considered to be one of the most promising techniques for the determination of underivatized sugars with sufficient electrical charges for detection and a wide range of polarities and molecular sizes, including both neutral monosaccharides and linear and branched polysaccharide [6]. In addition, the development of new stationary phases has improved liquid chromatographic methods enabling better separation of carbohydrates and carboxylic acids in a single chromatographic run [7]. With use of ion-exchange and size-exclusion LC, these two groups of compounds can be determined simultaneously in fruit [8], juices [9], cheese [10], and alcohol beverages [11].

The application of CE to the separation of neutral carbohydrates is discussed in the review articles of Paulus and Klockow [12], Honda [13], and El Rassi [14]. It is noted that in CE, as in gas and liquid chromatography, derivatization before separation is a common way to enhance sensitivity in

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carbohydrate analyses since carbohydrates form both ionic and absorptive/fluorescent species [12–15].

Carbohydrates exhibit weakly acidic properties and are totally ionized only in strongly alkaline solutions (pK_a 12–13) (Table 1). Analyses in highly alkaline solutions typically are done in sodium hydroxide at concentration of 10–100 mM [16–22].

Alkaline phosphate solutions were used by Carvalho *et al.* [16], and the method was optimized to enhance resolution and improve baseline stability. Direct UV detection in carbohydrate analyses by CE was achieved by in-capillary complex formation with borate. Complex formation increases the absorption of mono- and disaccharides at 195 nm [23]. Complexation can also be achieved by complex formation with copper(II) ion in alkaline separation medium [24].

Indirect detection based on a BGE containing chromophores for UV or fluorophores for LIF detection can be accomplished with a basic BGE to ionize the analytes [25–29].

Electrochemical detection is an interesting alternative to conventional detection in CE, because of the many other organic compounds than carbohydrates that are present in foods and organic solvents absorb UV light strongly. D-(+)-Glucose, D-(−)-fructose and sucrose in soft drinks, isotonic beverages, fruit juice, and sugar cane drinks have also been analyzed by CE with contactless conductivity detection [16]. Klampfl and Buchberger [30] have presented a method for the determination of underivatized carbohydrates using CE with detection by ESI-MS. They used highly alkaline electrolyte solutions prepared of volatile organic bases such as diethylamine (DEA). The volatility of the electrolyte solution allowed the CE technique to be combined with MS detection in negative-ion mode. The suitability of this method for the analysis of real samples was demonstrated with wine samples.

The aim of the present study was to develop and optimize a capillary zone electrophoretic method for the determination of mono- and disaccharides. The method relies on enediolate formation in a medium of extreme alkalinity. Identification was by direct UV detection. The applicability of the method is demonstrated in an analysis of selected sugars in beverage samples.

Table 1. The pK_a values of selected carbohydrates

Compound	MW (g/mol)	pK_a	Ref.
Arabinose	150.1	12.43	25
Fructose	180.2	12.03	25
Galactose	180.2	12.35	25
Glucose	180.2	12.35, 12.28[a)]	25, 26
Maltose	342.3	11.94	25
Mannitol	182.2	13.50[b)]	15
Mannose	180.2	12.08	25
Sucrose	342.3	12.51	25
Xylitol	150.1	13.8[c)]	31
Xylose	150.1	12.29, 12.15	25, 26

The ionization of carbohydrates occurs in C1.
a) Measured in water at 25°C.
b) Measured in water at 18°C.
c) Measured in water at 22.5°C.

2 Materials and methods

2.1 Materials

D-(+)-Sucrose, D-(+)-xylose, D-(+)-cellobiose, D-(+)-galactose, D-(+)-fucose, and D-(+)-mannose were purchased from Fluka (Buchs, Switzerland). L-Rhamnose monohydrate was from Sigma (St. Louis, MO, USA), and D-(+)-glucose, D-(−)-fructose, D-(−)-mannitol, xylitol, disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), and glacial acetic acid were from Merck (Darmstadt, Germany). D-(−)-Arabinose and D-(−)-ribose were from J. T. Baker Chemicals (Deventer, Holland) and Hoffman – La Roche (Basel, Switzerland), respectively. Aqueous solution of 1 M sodium hydroxide (NaOH) was purchased from FF-Chemicals (Haukipudas, Finland). Aqueous solution of 1 M potassium hydroxide (KOH) was prepared from Titrisol concentrate (Merck). All chemicals were used as received. Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2 CE procedures

Separations of carbohydrate standards and juice samples were carried out with a P/ACE MDQ CE instrument (Beckman-Coulter, Fullerton, CA, USA). Detection was done with direct UV monitoring using a photodiode array detector at wavelength 270 nm with a bandwidth of 10 nm.

Uncoated fused-silica capillaries of 50 μm id and length 50/60 cm (effective length/total length) were employed. Both the capillary and samples were thermostatted to +15°C. The samples were injected with a pressure of 0.5 psi for 4 s. After sample injection, a small portion of the electrolyte solution was injected with the same injection pressure for 5 s. The separation voltage was raised linearly within 2 min from 0 to +16 kV. The studied voltage range was 10–25 kV.

The new capillaries (from Teknolab AS, Trollåsen, Norway) were conditioned by rinsing with 0.1 M sodium hydroxide (20 min), Milli-Q water (20 min), and the electrolyte solution (20 min). Between analyses the capillaries were rinsed with 10% v/v acetic acid at a pressure of 15 psi for 4 min and with electrolyte solution at a pressure of 20 psi for 5 min.

2.3 Measurement of pH

The pH value of the electrolyte solution was measured with a Denver model 20 pH meter with combination electrode (Denver Instrument, Denver, CO, USA) calibrated with commercial buffers of pH 7.00 (±0.01), 10.00 (±0.01), and

12.00 (±0.01) (Reagecon, Shannon, Ireland, and Reagena Oy, Finland).

2.4 Carbohydrate standard solutions

For the method development and quantification, 50 mM stock solutions of the individual carbohydrates were prepared in Milli-Q water. The stock solutions were stored in a refrigerator (+4°C). Working standard solutions with a concentration range of 0.01–2 mM for capillary electrophoretic studies were prepared by appropriate dilutions of the stock solutions with Milli-Q water. Methanol (1%) in water was used as the neutral marker and it was added to standard solutions.

The detection limits were determined at S/N of 3 for all analytes.

The standard solutions with a carbohydrate concentration range between 0.25 and 4 mM for quantitation of neutral carbohydrates sucrose, D-(+)-glucose, and D-(−)-fructose in fruit juices and in alcoholic beverage were prepared by diluting stock solutions with Milli-Q water.

2.5 Sample solutions

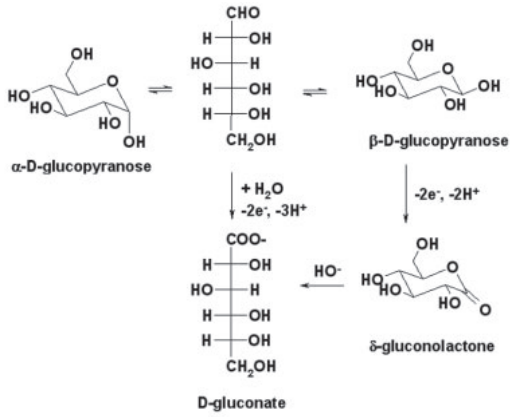
Three fruit juices, orange (Valio, Helsinki, Finland), pineapple (Marli, Turku, Finland), and lemon (Sicilia®, imported by Suomen Täysmarkkinointi Oy), were purchased from a local market. A cognac sample (Claude Chatelier XO, France) was purchased from a tax-free shop in a Viking Line ship (Viking Line Abp, Maarianhamina, Finland). Prior to analysis the juices were diluted to 1:50 v/v or 1:100 v/v and the cognac sample to 1:10 v/v with Milli-Q water. All the carbohydrate standards and samples were analyzed in triplicate.

2.6 Preparation of the carrier electrolyte solution

The electrolyte solution was prepared by mixing 450 mM stock solution of disodium hydrogen phosphate dihydrate with 1 M NaOH solution. Other solutions used in the electrophoresis were 130 mM NaOH and 130 mM KOH solutions. The latter was prepared by dilution of the 1 M KOH stock solution. All electrolyte solutions were filtered through 0.45 μM disposable filters (GHP Acrodisc, Gelman Sciences, Ann Arbor, MI, USA) prior to the analyses.

3 Results and discussion

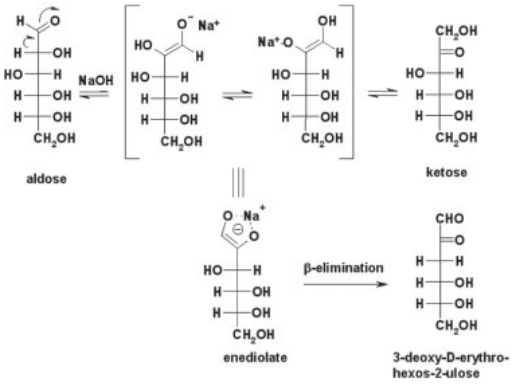
Several studies describing the oxidation mechanisms for carbohydrates in alkaline media are found in the literature [31, 32]. The main oxidation products of carbohydrates are C3–C6 acids. For example, the oxidation of D-(+)-glucose in basic medium leads selectively to the formation of the δ-gluconolactone, which undergoes hydrolysis forming gluconic acid as the final product (Scheme 1) [31–33].



Scheme 1. Oxidation of D-(+)-glucose to D-gluconate in alkaline medium [32, 39].

In aqueous alkaline solution, carbohydrates may participate in a reaction cascade of ionization, mutarotation, enolization, and isomerization resulting in the formation of enediolate anion. The pH of our electrolyte solution is 12.6, which means that all the carbohydrates were partially ionized. The ionization occurs on the C-1 hydroxy group because the acidity of the hemiacetal hydroxy group (Table 1) is stronger than that of the alcoholic hydroxy group ($pK_a = 16$) [34]. The enolization reaction is part of the Lobry de Bruyn–Alberda van Ekenstein rearrangement [35–39]. The enediolate species involved in the rearrangement are considered to be intermediates in the alkaline degradation of carbohydrates, which produces organic acids.

The proposed UV-absorbing anion is the carbohydrate enediolate. A tentative reaction mechanism for the formation of the enediolate is presented in Scheme 2. The formal intramolecular redox reaction, or 1,2-proton shift, com-



Scheme 2. Proposed reaction mechanism for enediolate formation and the subsequent β-elimination reaction [37–39].

mences with the abstraction of proton from the C-2 atom of aldose and is followed by the formation of an enediolate intermediate. Finally, the C-1 atom of the carbohydrate-derived enediolate is protonated to yield the corresponding ketose. Ketoses equilibrate to aldoses in alkaline solution *via* enediolate formation. Sodium cation present in the electrolyte solution is able to interact with the enediolate anion, preventing the reaction from proceeding to the carboxylic acid products.

The typical alkaline degradation products of glucose and other hexoses possess conjugated enol carbonyl that generates UV absorption at 265 nm [38]. In addition, the enediolate can participate in β-elimination reaction yielding α-dicarbonyl compound. For example, the enediolate derived from glucose may undergo β-elimination to give 3-deoxy-D-*erythro*-hexos-2-ulose with UV absorption at 265 ± 5 nm.

Presumably, direct UV detection of our carbohydrates is possible due to the absorption of the conjugated carbonyl groups (C=O) of 3-deoxy-D-*erythro*-hexos-2-ulose. Absorption occurs in strongly alkaline conditions. Under our optimized electrolyte conditions the absorption peak of carbohydrates at around 270 nm exhibits molar absorptivity of about 10 [40] (Fig. 1).

Most probably, similar degradation mechanism to that of Scheme 2 was exploited by Roig *et al.* [41, 42] in the determination of sugars in wine and juices. Photodegradation in alkaline solution yielded a by-product that was detectable with a UV detector at 268 nm.

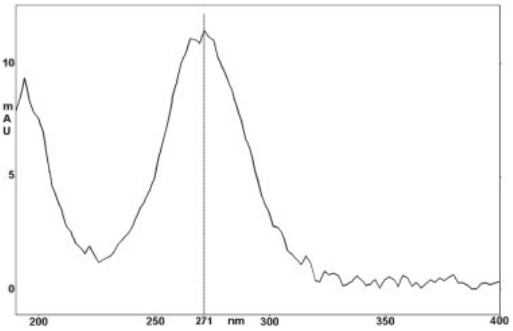


Figure 1. UV spectrum of 1 mM glucose in the range of 190–400 nm recorded in optimized separation electrolyte, 130 mM NaOH–36 mM $Na_2HPO_4 \cdot 2H_2O$, pH 12.6.

3.1 Effects of hydroxide and phosphate concentrations on resolution and migration time

The optimized electrolyte contained 130 mM NaOH and 36 mM $Na_2HPO_4 \cdot 2H_2O$. The pH value and the ionic strength of the electrolyte were 12.6 and 0.217 M, respectively, creating a current of 145 μA and separation power of 3.5 W/m under 16 kV separation voltage. Although our studies showed the UV signal of enediolate to be the strongest at sodium hydroxide concentrations between 70 and 100 mM, a stronger sodium hydroxide concentration was chosen to decrease the mobility and improve resolution [17, 18]. The addition of disodium hydrogen phosphate to the electrolyte solution resulted in an improvement in resolution and less noisy baseline. The same was noticed by Carvalho *et al.* [16]. Most probably, its role was only to increase the viscosity of the electrolyte solution and reduce the mobilities. Our results did not show any enhanced sensitivity in the phosphate buffered solution.

The optimized concentrations of NaOH and disodium hydrogen phosphate dihydrate gave the best resolution between glucose, L-rhamnose, mannose, and arabinose. Moreover, the total analysis time was less than 40 min.

Potassium hydroxide of similar concentration to the sodium hydroxide was tested for pH adjusting of the electrolyte solution. KOH-based electrolyte solutions with and without phosphate addition were investigated. Carbohydrate separations occurred faster in pure KOH, but the resolution of the analytes was poorer. With sodium hydroxide alone the good peak resolution vanished. Colón *et al.* [17] attributed the difference in mobilities in NaOH and KOH to the sphere of solvation of the alkali metal cation. The ratio of ionic charge to ionic radius decreases as the size of the alkali metal increases. Sodium has a larger sphere of hydration than potassium. This effect reduces the mobility of carbohydrates under the influence of an electric field, which in turn produces a longer analysis time. Furthermore, under identical experimental conditions the separation current was slightly higher in electrolyte solutions containing KOH than in those containing NaOH (Fig. 2).

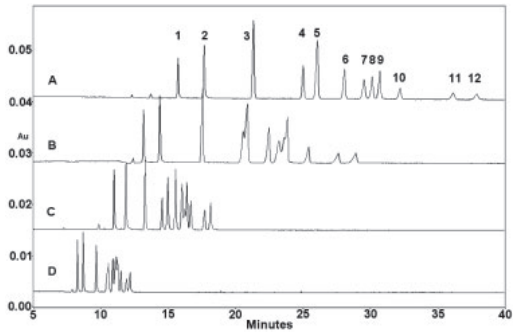


Figure 2. Comparison of four alkaline electrolytes in the analysis of 12 carbohydrates, 1 mM each. Peak identities: 1, xylitol; 2, mannitol; 3, sucrose; 4, fucose; 5, cellobiose; 6, galactose; 7, glucose; 8, rhamnose; 9, mannose; 10, arabinose; 11, xylose; 12, ribose. Separation conditions: +16 kV; detection, 270 nm direct mode; injection pressure, 0.5 psi for 4 s; capillary, 50/60 cm (L_{det}/L_{tot}); separation temperature, 15°C. Compared electrolytes, separation current and pH of the electrolyte during analysis: (A) 130 mM NaOH–36 mM $Na_2HPO_4 \cdot 2H_2O$ (140 μA, pH 12.6); (B) 130 mM NaOH (155 μA, pH 12.7); (C) 130 mM KOH–36 mM $Na_2HPO_4 \cdot 2H_2O$ (150 μA, pH 12.8); (D) 130 mM KOH (165 μA, pH 13).

Some generalizations can be made in regard to the migration order of the carbohydrates. The first group contains sugar alcohols, which migrate immediately after the electroosmosis. Disaccharides migrate before aldohexoses, which in turn move faster than aldopentoses. Furthermore, 6-deoxy carbohydrates migrate faster than their parent compounds, *i.e.*, 6-deoxy-D-galactose [D-(+)-fucose] migrate faster than galactose and 6-deoxy-L-mannose [L-rhamnose] faster than mannose.

3.2 Effect of instrumental parameters on resolution and migration

Resolution was enhanced slightly when the total length of the capillary was increased from 60 to 80 cm, but the mobility was lower. The increased migration time with longer separation capillary was poorly compensated with higher separation voltage or higher temperature due to the lower resolution, increased current, and increased heat dissipation. Separation voltage was optimized to +16 kV. In that case the separation current was 145 μA in 130 mM NaOH–36 mM $Na_2HPO_4 \cdot 2H_2O$. The slow increase of the voltage gradient, at a rate of 7.5 kV/min, was observed to be favorable for method repeatability [43]. As expected, at +25°C the current fluctuation and increased Joule heating weakened the repeatability of the analyses. Furthermore, the resolution was diminished between glucose, rhamnose, mannose, and arabinose. Hence, the temperature was decreased to +15°C.

Two other technical aspects that needed to be considered were rinsing with the electrolyte and buffer depletion. If only one rinsing with electrolyte was applied in the method, the resolution between mannose and arabinose was diminished and the current was decreased by more than 10 μA in consecutive analyses. Both effects were clearly observed when ten or more samples were run consecutively. When a rinse with 10% acetic acid was introduced the problems were avoided and the repeatability of the analyses was much improved. The resolution between mannose and arabinose was restored to the level of the first run and the current was stable. After this improvement the performance of a capillary was excellent during a 3-week period.

Buffer depletion was observed during the method development, most strongly with alkali concentrations of 75–100 mM at ionic strengths 0.16–0.19 M. The depletion was expressed as a decrease in both peak area and height in two successive runs but stabilizing to a certain level after three or more analyses. In fact, the phenomenon had no effect on the electrophoretic mobilities and changes in both peak area and height could be compensated by using internal standard in the quantification. Our experiment showed that the injection of a short plug of the electrolyte solution after the sample injection followed by a slow increase in the separation voltage had a positive effect on the repeatability, which was best when fresh electrolytes was employed for every analysis. The same has been noted elsewhere [43, 44]. In view of the buffer depletion and to ensure good reproducibility, fresh electrolyte was used for each analysis.

3.3 Calibration, detection limits, and reproducibility

The calibration curve for each carbohydrate was prepared with six parallel analyses of eight sugar mixtures ranging in concentration from 0.05 to 2 mM. The linearity and sensitivity were determined for 11 carbohydrates, with D-(+)-fucose (1 mM) used as internal standard. The detection limits (at S/N of 3) were 0.02 mM for xylitol, mannitol, sucrose, fucose, cellobiose, galactose, glucose, and rhamnose and 0.05 mM for arabinose, xylose, and ribose.

The effective mobility, μ_e, for each carbohydrate was calculated using Eq. (1) [26]:

$$\mu_e = \frac{L_{det} L_{tot}}{t_R V} - \frac{L_{det} L_{tot}}{t_{EOF} V} \ [m^2 V^{-1} s^{-1}] \quad (1)$$

where L_{det} and L_{tot} are the length of the capillary to the detector and the total length of the capillary, respectively. V is the applied potential, t_R is the migration time of the anion, and t_{EOF} is the migration time of methanol used as a neutral marker. The effective mobilities of the 12 sugars determined by the presented method are listed in Table 2. The results

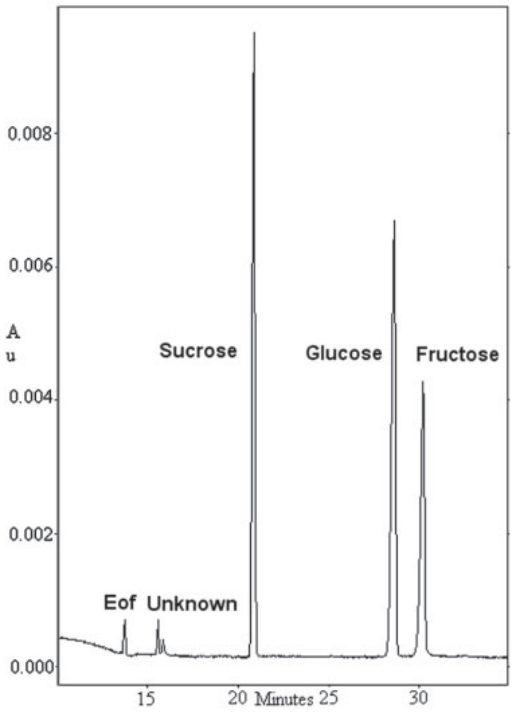


Figure 3. Electropherogram of orange juice. Sample was diluted 1:50 v/v with water. Separation conditions: +16 kV; detection, 270 nm direct mode; injection pressure, 0.5 psi for 4 s; capillary, 50/60 cm (L_{det}/L_{tot}); separation temperature, 15°C. Electrolyte: 130 mM NaOH–36 mM $Na_2HPO_4 \cdot 2H_2O$, pH 12.6.

Table 2. Linearity, precision, and mobilities of the studied carbohydrates at pH 12.6

Sugar	Calibration curves				RSD% (n = 6)			
	Without ISTD	Correlation coefficient	With ISTD	Correlation coefficient	Absolute migration time	Absolute peak area	Relative peak area	Mobility ($\times 10^{-8}$ m^2V^{-1} s^{-1})
Xylitol	$y = 23\,143x - 593.95$	0.972	$y = 0.8299x - 0.0494$	0.997	0.33	7.3	7.5	−0.278
Mannitol	$y = 37\,349x - 1623.8$	0.972	$y = 1.3172x - 0.0711$	0.993	0.43	8.5	5.8	−0.488
Sucrose	$y = 42\,191x + 3194.9$	0.941	$y = 1.5039x + 0.0643$	0.989	0.7	7.3	3.5	−0.772
Fucose					0.77			−1.001
Cellobiose	$y = 47\,520x - 947.83$	0.968	$y = 1.6938x - 0.0795$	0.998	0.73	7.8	3.2	−1.039
Galactose	$y = 42\,466x - 6233.5$	0.973	$y = 1.5137x - 0.2371$	0.96	0.84	10.5	7.3	−1.119
Glucose	$y = 36\,038x - 5127.2$	0.979	$y = 1.3034x - 0.216$	0.962	0.82	11.5	8.8	−1.176
Rhamnose	$y = 31\,494x - 4934.7$	0.972	$y = 1.1352x - 0.2074$	0.965	0.72	10.3	6.5	−1.200
Mannose	$y = 48\,628x - 9878.4$	0.981	$y = 1.696x - 0.3094$	0.96	0.7	9.7	10.5	−1.222
Arabinose	$y = 30\,718x - 5579$	0.964	$y = 1.1115x - 0.2272$	0.943	1	10.8	8.7	−1.257
Xylose	$y = 16\,067x - 3154.9$	0.972	$y = 0.5829x - 0.1306$	0.956	1	13.3	7.7	−1.365
Ribose	$y = 16\,673x - 2535$	0.984	$y = 0.606x - 0.1142$	0.973	1.05	12.1	11.2	−1.402

Fucose at a concentration of 1 mM was used as the internal standard (ISTD).

Table 3. Quantitative results for sucrose, glucose, and fructose in fruit juices and cognac

Juice	Sucrose (mM)	Glucose (mM)	Fructose (mM)	Total (g/L)	Total sugar content in product specification (g/L)
Lemon	——[a)]	41	38	14	—[b)]
Orange	128	149	119	92	110
Pineapple	150	163	116	101	120
Cognac	4.9	6.4	6.9	4.1	—[b)]
Calibration range (mM)	0.5–3	0.25–3	0.25–3		
Calibration curve	$34521x + 19579$	$44302x - 7682.9$	$29629x - 6210.7$		
Correlation coefficient (R^2)	0.9928	0.9939	0.9976		

a) Not detected.
b) Not announced.

show that the reproducibility of the absolute migration times is good, and the reproducibility of the absolute peak areas is on a satisfactory level.

3.4 Analyses of beverage samples

The main sugar components of three fruit juices and one cognac sample were determined. The carbohydrates measured were sucrose, glucose, and fructose. Figure 3 presents the electropherogram of orange juice as an example of the analyses of fruit juices. The three quantified carbohydrates can be seen as well as two small signals of unidentified compounds. The quantitative results are compiled in Table 3. Good agreement with the product specification was observed. The LOD and LOQ were 0.01 and 0.02 mM for sucrose and 0.02 and 0.05 mM for glucose and fructose, respectively.

4 Concluding remarks

Strong alkaline electrolyte can be considered an exceptional solution for the separation of neutral mono- and disaccharides. UV detection at wavelength 270 nm was highly

sensitive for all the carbohydrates presented here, without the need for derivatization. In excess of 150 real samples could be analyzed for sucrose, glucose, and fructose without detrimental effect on the capillary surface.

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PAPER II

Determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis

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Determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis

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Abstract

The neutral sugar composition of acid hydrolyzed extracts of cellulose fiber samples, i.e. oat spelt, wheat straw, thermomechanica pulp (TMP) made of spruce, aspen stemwood, and bleached birch kraft pulp, was determined by a new capillary zone electrophoresis (CZE) method employing an alkaline background electrolyte. The method relies on in-capillary reaction and direct UV detection at wavelength 270 nm. Neutral carbohydrates D-(+)-galactose, D-(+)-glucose, L-rhamnose, D-(+)-mannose, D-(−)-arabinose, and D-(+)-xylose were simultaneously separated. The calibration plots were linear over a range from 10 to 150 mg/L for D-(+)-galactose, L-rhamnose, D-(+)-mannose, and D-(−)-arabinose and from 50 to 400 mg/L for D-(+)-glucose and D-(+)-xylose. Relative standard deviations (RSDs) of peak areas during a 5-day analysis period varied from 3.3% for galactose to 11.8% for rhamnose. RSDs of migration times varied between 0.3 and 0.7%. The detection limit (at S/N 3) was 5 mg/L for each monosaccharide. The results obtained by CZE agreed well with results obtained by high-performance anion-exchange chromatography. Glucose and xylose were the two predominant monosaccharides in the plants, except in the spruce TMP sample where glucose and mannose dominated.

Keywords: Capillary electrophoresis; Monosaccharide; Hydrolysis; Plant; Direct UV detection; Wheat straw; Oat spelt; Spruce TMP; Aspen; Birch kraft pulp

1. Introduction

Knowledge of the role and behavior of polysaccharides, specifically cellulose and hemicelluloses, during wood and pulp processing is essential for understanding and controlling the processes [1]. Polysaccharide components that retain lignin in chemical pulp fibers during cooking and bleaching have been suggested to hinder the removal of the lignin from fibers. Moreover, acidic polysaccharides may become enriched in the circulation water of paper machines, interacting with the dissolved and colloidal substances in process waters. Identification of the polysaccharide composition is often desired to estimate the quality of the product.

The main structural components of wood are cellulose, hemicellulose, and lignin, together with a smaller amount of pectic substances (Table 1) [2]. Cellulose is a linear homopolymer of D-glucose units, cross-linked with β-(1–4)-glycosidic groups, and is the main polysaccharide in wood [3]. Hemicelluloses are heteroglycans with a backbone composed of β-1,4 -linked glucose, mannose, xylose, and galactose. The content and composition of hemicelluloses vary with the type of wood, as shown in Table 1. Galactoglucomannan, glucomannan, and arabinoglucuronoxylan are the major hemicelluloses in softwood (coniferous tree), accompanied by smaller amounts of arabinogalactan, xyloglucan, other glucans, and pectins. The main hemicelluloses of hardwood (deciduous tree) are glucuronoxylan and glucomannan [1].

The carbohydrate composition of polysaccharides of different origins can be determined after their hydrolysis to neutral monosaccharides with acids or enzymes [4]. Two-stage acid hydrolysis is a widely used pretreatment method, in which the fine-ground material is first swelled and dissolved in concentrated (approximately 70%, v/v) sulfuric acid and then hydrolyzed again in the dilute (1 M) mixture at elevated temperature (100–120 °C). Fairly harsh conditions are needed to break the covalent bonds of the monosaccharide units in cellulose and to destroy the glycosidic bonds of lignin–carbohydrate complexes [5,6].

The liberated monosaccharides can be separated and quantified by chromatographic methods such as gas chromatography (GC), high-performance liquid chromatography (HPLC),

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Table 1
The structural components of softwood and hardwood, presented as percent of dry wood [3]

Type of wood	Cellulose (%)	Hemicelluloses (%)		Other polysaccharides (%)	Lignin (%)
		Glucomannan	Xylan		
Softwood	33–42	14–20[a]	5–11[b]	3–9	27–32
Hardwood	38–51	1–4[c]	14–30[d]	2–4	21–31

[a] Galactoglucomannan: galactose-rich fraction and galactose-poor fraction (glucomannan).
[b] Arabinoglucuronoxylan.
[c] Glucomannan.
[d] Glucuronoxylan.

and high-performance anion-exchange liquid chromatography (HPAEC) [4]. In GC, neutral carbohydrates are derivatized by silylation or acetylation before analysis [5–10], whereas in HPAEC pulsed amperometric detection (PAD) is used to identify the underivatized monosaccharides [11,12]. Size-exclusion chromatography (SEC) has been applied to determine the molar mass distributions of cellulose and hemicelluloses [7,9,10,13]. Among modern techniques, NMR spectroscopy has proved useful in elucidating the structural details of polysaccharides [8,9,11,13].

Recently, capillary electrophoretic (CE) methods have been introduced to quantify neutral carbohydrates [14–16]. Carbohydrates contain hydroxyl groups that are ionized only in strongly alkaline conditions. After ionization, the structures are suitable for electrophoretic analysis and identification through indirect UV detection [17–21] or electrochemical detection [22–24]. In experiments on cellulosic fibers [5,25], an alkaline borate buffer was used as the electrolyte solution, and neutral carbohydrates were derivatized in a reductive amination reaction with 4-aminobenzonitrile or 4-aminobenzoic acid before CE analysis. Derivatization allows a specific monitoring wavelength to be applied to enhance detection sensitivity, and electrolyte solutions of moderate alkalinity are usually adequate. Although the derivatization of carbohydrates leads to improved sensitivity and resolution, the complexity of derivatization has disadvantages, such as recovery problems in quantification.

Direct UV detection of carbohydrates in CE has commonly been achieved by in-capillary complex formation with borate, which increases the UV absorption of mono and disaccharides at wavelength 195 nm [26]. In a previous paper, we presented a capillary zone electrophoretic (CZE) method where direct UV detection was successfully applied in the analysis of neutral mono and disaccharides and sugar alcohols in beverage samples [27]. The method is based on the formation of the anionic carbohydrate enediolate through the concomitant action of the alkali of the electrolyte solution and the applied electric field.

The aim of the present study was to evaluate the suitability of CZE separation for the quantification of neutral monosaccharides in cellulosic fibers of different origin liberated by acid hydrolysis. The method relies on in-capillary enediolate formation in an alkaline electrolyte solution and detection of the anion formed by direct UV absorption. This is the first report where the formation of enediolate anion is exploited to monitor monosaccharides in hydrolyzed plant fibers.

2. Materials and methods

2.1. Materials

D-(+)-Xylose, D-(+)-galactose, and D-(+)-mannose were purchased from Fluka (Buchs, Switzerland). L-Rhamnose monohydrate was from Sigma (St. Louis, MO, USA), and D-(+)-glucose, disodium hydrogenphosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$), and glacial acetic acid were from Merck (Darmstadt, Germany). D-(−)-Arabinose was from J.T. Baker (Deventer, The Netherlands). Aqueous solution of 1 M sodium hydroxide (NaOH) was purchased from FF-Chemicals (Haukipudas, Finland). All chemicals were used as received. Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Table 2 presents molecular formulas and weights, dissociation constants, and chemical structures of the monosaccharides.

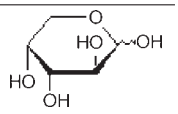
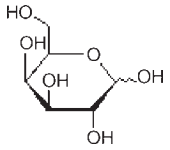
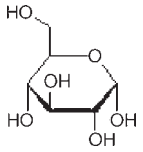
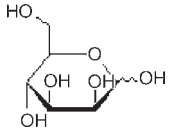
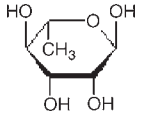
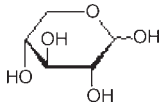
2.2. Pretreatment of plant samples

Oat spelt, wheat straw, spruce thermomechanical pulp (TMP), aspen stemwood, and bleached birch kraft pulp samples were hydrolyzed with sulfuric acid at VTT (Espoo, Finland) [28]. Five parallel hydrolyzed samples of each sample type were prepared. Exceptionally, before the hydrolysis treatment, spruce TMP and aspen samples were extracted with acetone–water mixture (95:5, v/v) to remove lipophilic compounds. All fiber samples were air dried, ground, and extracted twice. The prehydrolysis was performed with 70% (v/v) sulfuric acid at 30 °C for 1 h, after which the sample was diluted with water and hydrolysis was continued in a 2.4% (v/v) sulfuric acid solution at 120 °C for 50 min. Finally, the samples were cooled to room temperature, diluted with water, and percolated through 0.45 μm disposable filters (GHP Acrodisc, Gelman Sciences, Ann Arbor, MI, USA). The hydrolyzed samples were analyzed by CZE both without dilution and after dilution to 1:3 (v/v) with Milli-Q water. The diluted samples were used for quantitation of glucose and xylose and the undiluted samples for quantification of the other monosaccharides.

2.3. Preparation of the carrier electrolyte and monosaccharide standard solutions for CZE analyses

An electrolyte solution consisting of 130 mM NaOH and 36 mM $Na_2HPO_4{\cdot}2H_2O$, was prepared by mixing 450 mM stock solution of disodium hydrogenphosphate dihydrate with 1 M

Table 2
Molecular formulas, molar masses, pK_a values, and chemical structures of the monosaccharides studied

Compound	Molecular formula	MW (g/mol)	pK_a[a]	Chemical structure
D-(−)-Arabinose	$C_5H_{10}O_5$	150.1	12.43	
D-(+)-Galactose	$C_6H_{12}O_6$	180.2	12.35	
D-(+)-Glucose	$C_6H_{12}O_6$	180.2	12.35	
D-(+)-Mannose	$C_6H_{12}O_6$	180.2	12.08	
L-Rhamnose	$C_6H_{12}O_5$	164.2	NA[b]	
D-(+)-Xylose	$C_5H_{10}O_5$	150.1	12.29	

[a] From Ref. [18].
[b] Not available.

sodium hydroxide solution. The electrolyte solution, having a pH value of 12.6 and ionic strength of 0.217 M, was led through 0.45 μM disposable filters (GHP Acrodisc). Before the analyses it was sonicated for 20 min at room temperature.

The pH value of the electrolyte solution was measured with a Denver model 20 pH meter equipped with a combination electrode (Denver Instrument Co., Denver, CO, USA). The electrode was calibrated using a customer-defined calibration range employing commercial buffers of pH 7.00 (±0.01), 10.00 (±0.01), and 12.00 (±0.01) (Reagecon, Shannon, Ireland, and Reagena, Toivala, Finland).

Stock solutions of 10 000 mg/L were prepared for each carbohydrate. For arabinose, mannose, rhamnose, and galactose, working standard solutions with a concentration range of 5–150 mg/L were made by appropriate dilutions of the stock solutions with Milli-Q water.

For glucose and xylose concentrations were 50–400 mg/L. The solutions were stored in a refrigerator (+4 °C). The detection limits of analytes were determined at signal-to-noise ratio of 3. Two parallel measurements were performed on each standard and sample.

2.4. Capillary electrophoresis methods

Separations of the carbohydrate standards and acid hydrolysis samples were performed with a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a photodiode array UV detector. Monitoring of carbohydrates was carried out using direct UV detection at wavelength 270 nm with a bandwidth of 10 nm.

Uncoated fused silica capillaries of 50 μm I.D. and length 50/60 cm (effective length/total length) were employed in the experiments. Both the capillary and samples were thermostated to +20 °C. The samples were injected at a pressure of 0.5 psi (34.47 mbar) and the injection time was optimized to 6 s. A small portion of the electrolyte solution was then injected, while applying the same injection pressure for 5 s. The separation voltage was raised linearly within 1 min from 0 to +17 kV. Before the measurements, the new capillaries (from Teknolab Trollåsen, Norway) were conditioned by rinsing sequentially with 0.1 M sodium hydroxide, Milli-Q water, and the electrolyte solution, each solution for 20 min. Between analyses the capillaries were rinsed with the electrolyte solution at a pressure of 20 psi (1379 mbar) for 5 min. The signal of the system peak was used as marker for the electroosmotic flow (EOF) and it was checked by an injection of methanol [27].

2.5. Anion-exchange chromatographic method

HPAEC analyses were performed at VTT (Espoo, Finland). The quantitative analyses of the monosaccharides were carried out by HPAEC-PAD. A Dionex DX-500 series chromatographic system (Sunnyvale, CA, USA) was equipped with a CarboPak PA-1 column. The solvents used in the gradient elution system were water, 100 mM NaOH, 100 mM NaOH + 300 mM sodium acetate, and 300 mM NaOH. The flow rate was adjusted to 1 ml/min and the column temperature was set to 30 °C.

3. Results and discussion

3.1. Separation of monosaccharides

Fig. 1 shows an electropherogram of the mixture of the six monosaccharides studied under optimized separation conditions. The migration order of the monosaccharides is D-(+)-galactose, D-(+)-glucose, L-rhamnose, D-(+)-mannose, D-(−)-arabinose, and D-(+)-xylose, indicating that hexoses migrate before pentoses (see the structures of the monosaccharides in Table 2).

The most suitable electrolyte solution for the monosaccharides was one consisting of 130 mM sodium hydroxide and 36 mM aqueous sodium hydrogenphosphate, resulting in pH of 12.6. The two chemicals had slightly different roles in the optimization procedure. Namely, the higher concentration of

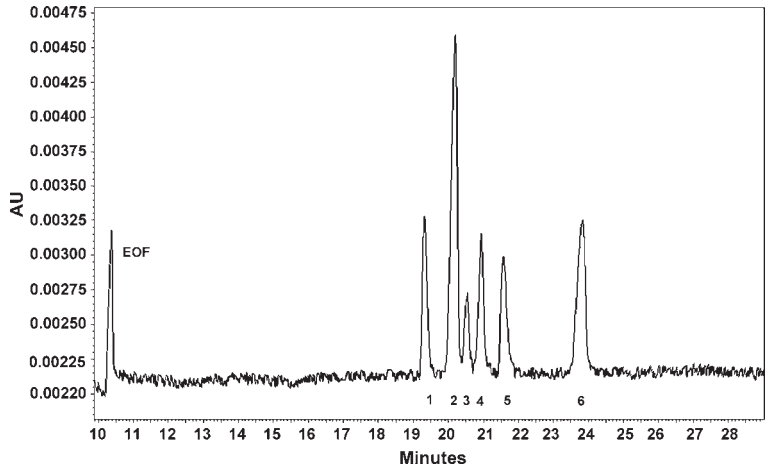


Fig. 1. Electropherogram of six monosaccharides in standard solution. The concentration of D-(+)-glucose and D-(+)-xylose is 300 mg/L and that of other monosaccharides 100 mg/L. Peak identification: 1, galactose; 2, glucose; 3, rhamnose; 4, mannose; 5, arabinose; 6, xylose. The peak of methanol indicates the electroosmotic flow (EOF). Separation conditions: +17 kV, detection at 270 nm with direct mode, injection pressure 0.5 psi for 6 s, capillary 50/60 cm (L_{det}/L_{tot}), separation temperature 20 °C. Electrolyte solution: 130 mM NaOH–36 mM $Na_2HPO_4 \cdot 2H_2O$ (pH 12.6).

sodium hydroxide in the solution improves the resolution among glucose, rhamnose, mannose, and arabinose by reducing the migration time of these analytes. The addition of disodium hydrogenphosphate to the electrolyte solution, in turn, not only increases the separation efficiency and resolution but also stabilizes the detection signal by decreasing the noise. Despite the chemical and instrumental optimizations, baseline resolution between glucose and rhamnose was not achieved, as is illustrated in Fig. 1.

The total analysis time including washing steps was about 30 min, which is undesirable for a method intended for use in routine analyses. Longer analysis time is excepted when the electrophoretic mobility of analytes is in opposite direction to the electroosmotic flow.

The repeatability of the analyses was markedly improved by acid rinsing of the capillary after each run. A rinse with 10% acetic acid stabilized the separation current to a fixed level during sequential analysis. The other favorable effect of organic acid was its ability to regenerate the capillary surface during the long analysis period [27].

The monosaccharides were identified by direct UV detection at wavelength 270 nm. The reaction that was employed has been presented in detail in our previous article [27]. As described there, in aqueous alkaline solution carbohydrates participate in a reaction cascade of ionization, mutarotation, enolization, and isomerization resulting in the formation of a UV absorbing anion. A proposal for the UV absorbing anion is the carbohydrate enediolate, which is a reaction intermediate in the oxidation of carbohydrates. Presumably, the direct UV detection of carbohydrates occurs through the absorption of the conjugated carbonyl groups (C=O) of 3-deoxy-D-*erythro*-hexos-2-ulose. The sodium cation present in the electrolyte solution is able to interact with the enediolate anion, preventing the reaction from proceeding to the carboxylic acid products. Similar degradation and epimerization reactions were observed by Roig and Thomas [29] and Lee [30].

3.2. Calibration, detection limits, and reproducibility

Linearity, precision, and regression equations for the first and last day of the analysis of standard mixtures of the carbohydrates are listed in Table 3. The results show excellent reproducibility of the absolute migration times, and acceptable reproducibility of the absolute peak areas.

A calibration curve for the carbohydrates was prepared for each hydrolysate of plant fiber with use of seven sugar concentrations. The calibrations were linear over the range from 10 to 150 mg/L for galactose, rhamnose, mannose, and arabinose and from 50 to 400 mg/L for glucose and xylose. With the seven different concentrations, relative standard deviations (RSDs) of peak areas varied from 3.3% for galactose to 11.8% for rhamnose for inter-day precision over a 5-day period, as shown in Table 3. For migration times, the corresponding value is between 0.3 and 0.7%. The detection limit (at S/N 3) was 5 mg/L with pressure injection of 0.5 psi (34.47 mbar) for 6 s for each monosaccharide.

3.3. Quantification of acid hydrolysis samples

Monosaccharide composition was determined for five different plant materials.

Oat spelt and wheat straw are fiber samples of annual plants. Spruce TMP represents softwood, while aspen stemwood and birch samples represent hardwoods. Spruce TMP and bleached birch kraft pulp are more highly processed samples than the others.

The standard method recommended for acid hydrolysis of extractive-free wood and pulp is a two-step hydrolysis with sulfuric acid [31]. The pre-hydrolysis step mostly releases

Table 3
Repeatability of the CZE analytical procedure: mean values, average relative standard deviations (RSD %), regression equations, and coefficients for five independent quantitations for the first and last days of the analysis of six monosaccharides in standard mixtures

Monosaccharide	Concentration range (mg/L)		Migration time[a]		Peak area[a] (Regression)		
			Mean (minute)	Average RSD %	Equation ($y = ax + b$)	Coefficient (r^2)	Average RSD %
D-(+)-Galactose	10–150	Day 1	19.41	0.6	$y = 109.42x + 231.73$	0.9964	5.7
		Day 5	19.35	0.5	$y = 116.06x + 186.59$	0.9964	3.3
D-(+)-Glucose	50–400	Day 1	20.13	0.4	$y = 85.064x + 3251.9$	0.9946	4.8
		Day 5	20.18	0.3	$y = 86.935x + 4995.4$	0.9971	3.7
L-Rhamnose	10–150	Day 1	20.48	0.4	$y = 45.623x - 128.12$	0.9967	11.8
		Day 5	20.54	0.3	$y = 60.465x - 341.51$	0.9906	9.0
D-(+)-Mannose	10–150	Day 1	20.90	0.6	$y = 98.478x + 23.839$	0.9974	10.1
		Day 5	20.96	0.4	$y = 105.55x - 23.801$	0.9997	7.9
D-(−)-Arabinose	10–150	Day 1	21.63	0.7	$y = 90.125x + 103.54$	0.9985	6.9
		Day 5	21.62	0.6	$y = 101.04x + 249.04$	0.9944	8.3
D-(+)-Xylose	50–400	Day 1	23.71	0.5	$y = 49.027x + 2724.7$	0.9907	5.1
		Day 5	23.80	0.4	$y = 60.512x + 1928.2$	0.9906	5.6

With injection of 6 s at 0.5 psi, the limit of detection (LOD) was 5 mg/L for all of the analytes.
[a] Seven concentration levels of standards, two injections/concentration level.

oligosaccharides from the lignocellulosic matrix, and the following post-hydrolysis step mainly cleaves the bonds between sugars in the released oligosaccharides. A drawback of the method is that some of the monosaccharides that are released may be destroyed during the hydrolysis [5]. Acid hydrolysis is nevertheless a highly efficient method to achieve the desired hydrolytic cleavage of glycosidic bonds in a cellulosic sample.

The quantitative results for neutral carbohydrates obtained by CZE are compiled in Table 4, with the results obtained by HPAEC-PAD included for comparison. The results for three parallel hydrolyzed samples show that L-rhamnose was not detected in any samples. This was due to the high limit of detection for the sugars (i.e. 5 mg/L) with the 6 s injection, as well as to peak overlapping. As also shown in Table 4, the results obtained by CZE and HPAEC-PAD are comparable in terms of quantification, but the RSDs of the peak areas are higher for CZE. The chemical degradation of the monosaccharides in alkaline solution and the matrix effect due to the acid in the samples are the most probable sources of the poor repeatability.

The CZE and HPAEC methods gave different quantification results for arabinose in spruce TMP, aspen, and bleached birch kraft pulp. In the case of the spruce TMP sample, the relatively high mannose amount interferes with the separation and detection of arabinose making their quantification inaccurate. Moreover, the presented CZE method was not optimized for the separation of enantiomers of monosaccharides. Therefore, L-arabinose which is present in softwood [1,7] and D-arabinose which was as the arabinose standard are shown as one peak in the electropherograms.

Fig. 2 presents the electropherograms of the undiluted acid hydrolyzed samples. Peaks were identified through a comparison of migration times with those of the standard mixtures and by spiking the sample with individual monosaccharides. The peak profiles of glucose and xylose appeared as non-ideal Gaussian,

Table 4
Neutral carbohydrates determined in five fiber samples after acid hydrolysis

Sample		mg/100 mg dry weight (RSD %)							Dry weight (%)
		L-Rhamnose	D-(−)-Arabinose	D-(+)-Galactose	D-(+)-Glucose	D-(+)-Xylose	D-(+)-Mannose	Total amount of monosaccharides	
Oat spelt	CZE	n.d.	1.9 (20)	0.8 (19)	27 (13)	25 (7.4)	n.d.	55.6	94.7
	HPAEC	<0.1	2.8 (3)	1.3 (3)	32 (1)	30 (3)	<0.5	66.7	
Wheat straw	CZE	n.d.	1.7 (30)	0.5 (11)	40 (4.3)	16 (7.9)	0.2 (19)	59	94.9
	HPAEC	<0.1	2.2 (9)	0.8 (8)	42 (8)	23 (9)	0.4 (10)	69.1	
Spruce TMP	CZE	n.d.	n.d.	2.0 (2.3)	49 (13)	5.5 (4.8)	14 (8.1)	70.6	94.2
	HPAEC	0.14 (7)	1.2 (2)	1.9 (3)	49 (4)	5.6 (3)	13 (2)	71.9	
Aspen stemwood	CZE	n.d.	1.1 (22)	0.4 (16)	48 (12)	18 (3.7)	1.9 (16)	68.8	94.7
	HPAEC	0.29 (3)	0.3 (3)	0.5 (2)	51 (1)	18 (1)	2.2 (3)	73.9	
Bleached birch kraft pulp	CZE	n.d.	1.0 (28)	n.d.	86 (16)	25 (11)	0.6 (11)	112	95.7
	HPAEC	<0.1	<0.1	<0.1	80 (2)	25 (1)	0.5 (4)	105.7	

Results were calculated from three and five parallel analyses by CZE and HPAEC, respectively.
n.d. = not detected.

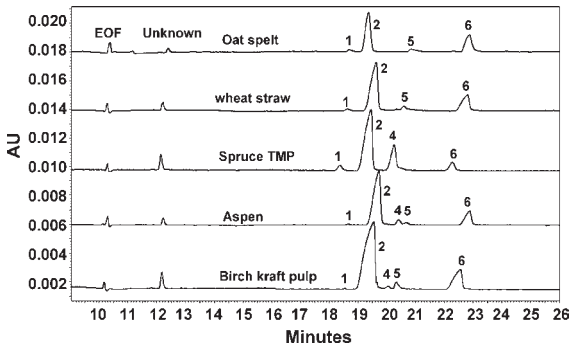


Fig. 2. Electropherograms of undiluted acid hydrolyzed samples. Peak numbering and separation conditions are as in Fig. 1.

which is a clear indication of overloading of the sample zone, and sample dilution was required for the more accurate quantitation. The undiluted samples were suitable for the analyses of galactose, mannose, and arabinose. Not all peaks were identified in the samples: an unidentified signal immediately after the signal of the electroosmotic flow appears in the electropherograms of all samples (Fig. 2) but not those of the standard solutions.

The electropherograms of oat spelt, wheat straw, and aspen stemwood samples are closely similar to the electropherogram of birch kraft pulp (Fig. 2). Glucose was the dominant monosaccharide in all samples, comprising 49.1–76.8% of the total neutral monosaccharides. Xylose was a major monosaccharide (22.4–45.7%) in all samples except spruce TMP, which exceptionally contained mannose (19.8%) in second largest amount. The primary source of glucose is cellulose, and this is directly reflected in the large amount of glucose of bleached birch kraft pulp, which is the most highly processed sample and contains almost 80% glucose. A minor portion of glucose comes from galactoglucomannan, which in softwood in one of the major hemicelluloses. Hardwoods also contain glucomannan, but in lesser degree than softwood (Table 1).

The monosaccharide content of wheat straw, measured by both CZE and HPAEC is in good agreement with the relative concentration ratios of 15:9:1 for glucose, xylose, and arabinose calculated by Durot et al. [12]. Calculations based on our results gave corresponding ratios of 23:9:1 for CZE and 19:10:1 for HPAEC method. The sugar composition of the spruce TMP was similar to earlier published data [32].

4. Concluding remarks

Our study shows that in-capillary reaction is a useful method for the analysis of neutral carbohydrates by capillary zone electrophoresis. UV detection at wavelength 270 nm was sensitive for five carbohydrates of interest. The benefit of the method is the non-required labelling of the analytes for the detection. The determination of galactose, glucose, rhamnose, mannose, arabinose, and xylose was done with use of a highly alkaline electrolyte solution containing sodium phosphate. The method is fast since only sample dilution is required in the sample preparation. The wide interest in the composition and concentration of carbohydrates in cellulosic fibers of different plants makes a selective method particularly appealing. The analysis of acid hydrolyzed samples of plant materials by CZE is comparable to the analysis by anion-exchange liquid chromatography, though the sample pretreatment step slightly reduces the quantitative precision and accuracy.

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PAPER III

Determination of the carboxylic acids in acidic and basic process samples by capillary zone electrophoresis

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Determination of the carboxylic acids in acidic and basic process samples by capillary zone electrophoresis

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ABSTRACT

Capillary zone electrophoresis (CZE) with indirect UV detection was used in developing a method for the simultaneous determination of inorganic anions, aliphatic and heterocyclic organic acids in various processed samples. The analytes were determined simultaneously in 10 min using an electrolyte containing 20 mM 2,3-pyrazine dicarboxylic acid, 65 mM tricine, 2 mM $BaCl_2$, 0.5 mM cetyltrimethylammonium bromide, and 2 M urea at pH 8.06. Linear plots for the analytes were obtained in the concentration range of 2–150 mg L^{-1}. Relative standard deviations (RSDs) of peak areas during a 3-day analysis period varied from 5.5% for glycolate to 9.5% for oxalate. RSDs of migration times varied between 0.4% and 1.1%. The detection limit (at S/N 3) was 1 mg L^{-1} for all the analytes studied. The proposed method was successfully demonstrated for the determination of carboxylic acids in eight oxygen treated samples of commercial softwood and hardwood kraft lignin and two red wine samples of Pinot Noir grapes. In the kraft lignin samples the concentrations of carboxylic acids correspond to the oxidation time. The acid concentrations of wine varied considerable.

1. Introduction

The analysis of organic acids and inorganic anions is of interest in many fields of industry and research, thus the demand for a simple and reliable method for the fast determination of anions has increased. A number of capillary electrophoretic separation methods for carboxylic acid have been published [1,2]. Generally, the common analytical set-up includes co-electroosmotic separation and indirect UV detection. A cationic surfactant has been added to reverse the electroosmotic flow and an UV absorbing organic aromatic or inorganic chromophore for monitoring non-absorbing analytes by the indirect UV detection mode. Moreover, while the indirect technique is not as sensitive as the direct one, good resolution of the analyte zones in the separation medium and narrow sample zones are needed to detect the low levels of the ions in real samples [3]. Adler et al. compared the chromophoric properties of 2,3-pyrazinedicarboxylic acid (2,3-PyDC) and 2,3-pyridinedicarboxylic acid (2,3-PDC) with the more common probe 2,6-pyridinedicarboxylic acid (2,6-PDC) [4]. The pH was adjusted above 10, where all the analyte are fully ionized. They observed that although the molecular structures were quite similar, the probes were different in respect of sensitivity. The applications of monitoring carboxylic acids by capillary electrophoretic methods cover a wide range of samples, including food and beverage [5–8] and industrial samples [9–13]. Although some CZE electrolytes based on the application of pyromellitic acid [14] or 2,6-pyridinedicarboxylic acid [7] as a chromophore have been commercialized as such or with some modifications [15,16], there is still room for BGE solutions for special purposes. For example, if the sample matrix is very acidic, basic, or highly ionic or the concentrations of the closely migrating analytes varies largely, it may be necessary to develop a new BGE solution to enhance resolution and sensitivity [12,17].

Chromatographic separation techniques offer competitive alternatives for analyzing organic acids of various matrices. Especially liquid chromatographic techniques include various possibilities in acid analyses [6,18–21]. Gas chromatography (GC) coupled with flame ionization (FID) or mass spectrometric (MS) detection offers a second alternative technique for the analysis of organic acids. This technique provides high detection sensitivity. However, most often GC methods require a complicated and time-consuming derivatization step, in which the acids are analyzed typically as trimethyl silyl (TMS) derivatives [22].

Wood contains cellulose, hemicellulose, and lignin. Their mutual portions are different in hardwood and softwood. The percentage portions of cellulotic compounds and lignin in dry wood varied from 52% to 85% and from 21% to 32%, respectively. The objective of the chemical pulping processes is to separate cellulose fibers from each other and to remove lignin from fibers [23,24]. The cooking chemicals react with the lignocellulose causing the lignin

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polymer to break down into smaller fragments [25,26]. At most 92–94% of the fiber lignin is dissolved during the bulk delignification phase and the residual lignin, attached to cellulose fibers, is removed in the following delignification and bleaching stages. The delignification of chemical pulp is often carried out with oxygen in alkaline medium. The oxidation reactions are targeted toward the phenolic structures in lignin. After delignification, the bleaching stages are needed to increase the pulp brightness [25,28].

Although it is important to study the chemical reactions in various stages of the pulping process, sidestreams of the forest industry can be potential raw material sources for the production of different industrially relevant products. The black liquor obtained from kraft cooking contains a rich mixture of inorganic anions, aliphatic and aromatic carboxylic acids, and phenolic compounds [13,21]. Typically, black liquor solution has been burned to obtain energy and for recycling sulfur anions, which are regenerated through the green liquor phase to white liquor [29]. However, the components of the cooking liquor can be used as a raw material for the production of chemicals [30].

Wine is a widely consumed beverage in the world with thousands of years of tradition.

Determining of authenticity of wine is important in respect to food quality and safety [18,31,32]. Red wine is a rather complex fluid containing for instance water, ethanol, acids, carbohydrates, and phenolic compounds [33]. Among other analyte groups, the total acid content affects the balance of the wine while the levels of an individual acid can impart flavor with direct effect on the taste [6,18,34,35].

The aim of this work was to develop a new background electrolyte (BGE) solution buffered with the zwitterionic tricine for the determination of closely related aliphatic carboxylic acids in strongly alkaline lignin samples. The oxidation of kraft lignin of hardwood and softwood produces a large amount of various carboxylic acids which can be used as raw material for new products. The concentrations of the formed carboxylic acids were analyzed as a function of the oxidation time. This data will be used for a more comprehensive study on the reaction kinetics of lignin oxidation. The applicability of the method was also tested in the determination of aliphatic carboxylic acid compositions in acidic samples. For this application, wine samples made of similar grape but different origins were selected.

2. Experimental

2.1. Materials

Na_2SO_4, $MgCl_2{\cdot}6H_2O$, sodium maleate, and glacial acetic acid were purchased from Sigma (St. Louis, MO, USA). Tricine, fumaric acid, and lactic acid come from BDH (Poole, UK). Glycolic acid, 2,3-pyrazinedicarboxylic acid (2,3-PyDC), succinic acid, and 2-furancarboxylic acid (2-furoic acid) were obtained from Fluka (Buchs, Switzerland). HCl (Titrisol), cetyl trimethylammonium bromide (CTAB), urea, $Na_2HPO_4{\cdot}2H_2O$, citric acid, and $BaCl_2{\cdot}2H_2O$, were purchased from Merck (Darmstadt, Germany). NaOH came from Akzo Nobel (Eka Nobel AB, Bohus, Sweden); formic acid and tartaric acid from Riedel- deHaën (France). Oxalic acid, malonic acid, and malic acid were obtained from Aldrich (Milwaukee, WI, USA).

All chemicals were analytical grade and used as received. Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and capillary electrophoresis method

Uncoated fused silica capillaries of 50 μm I.D. and length 50/60 (effective length/total length) were employed in the experiments. The capillary and samples were thermostatted to +15 and +20 °C, respectively. The samples were injected at a pressure of 0.5 psi (34.5 mbar) and the injection time was set to 10 s. The separation voltage was raised linearly within 1 min from 0 to −15 kV. Before the measurements, the new capillaries (from Teknolab Trollåsen, Norway) were conditioned by rinsing sequentially with 0.1 M sodium hydroxide, 0.1 M HCl and ultra pure water, each solution for 20 min and then with electrolyte solution for 5 min. Between analyses, the capillaries were rinsed with 0.1 M NaOH solution for 2 min and the electrolyte solution for 5 min, both using the pressure of 20 psi (1380 mbar).

Separations of the standards and samples were performed with a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a photodiode array (PDA) UV/vis detector. Monitoring of carboxylic acids was carried out using the indirect UV detection mode at wavelength 281 nm with a bandwidth of 10 nm.

2.3. Samples

Commercial softwood and hardwood lignin originated from kraft black liquor and were donated by MeadWestvaco (MWV, Corp., Appomattox, USA). These kraft lignins were dissolved in 0.1 M sodium hydroxide solution in concentration of 7.5 g L^{-1}. After dissolution samples were oxidized at a temperature of 90 °C. The initial oxygen pressure was set to 5 bars and the total reaction time was 240 min. Liquid samples were taken from a closed vessel through a sampling valve as a function of reaction time and the carboxylic acid quality and concentration were determined carefully with CE to reveal the degradation reaction kinetics. Prior to CE analyses, lignin samples were diluted to 1:10 (v/v) with ultra pure water.

Two red wine samples made of Pinot Noir grape from different continents and countries were purchased from a local liquor store. Wines were Pinot Noir Reserva del Fin del Mundo (Bodega del Fin del Mundo, Argentina, Patagonia 2007) and Pinot Noir du Valais (Cave St Pierre AC Valais, Switzerland 2007). Prior to analyses the wines were diluted 1:20 (v/v) with ultra pure water.

For quantitation, standards and samples were analyzed in triplicate. For method validation, four consecutive injections of standards were performed.

2.4. Standard solutions for CZE analyses

For method development and quantification, stock solutions of 10,000 mg L^{-1} in ultra pure water were prepared for each analyte. The working standard solutions with a concentration range of 1–150 mg L^{-1} were made by appropriate dilutions of the stock solutions with ultra pure water. The stock solutions were stored in a refrigerator (+4 °C), except fumarate, which was prepared in 40% methanol and stored in room temperature. The limit of detection (LOD, S/N of 3) was determined with standards in water, while the limit of (LOQ S/N of 10) was determined in matrix, for all the analytes.

2.5. Preparation of the electrolyte solution for CZE analyses

An optimized electrolyte solution consisted of 20 mM 2,3-PyDC, 65 mM tricine, 2 mM $BaCl_2$, 0.5 mM CTAB, and 2 M urea. Stock solutions of 100 mM of 2,3-PDC, tricine, CTAB, and barium chloride were prepared, and a suitable volume of each component was added to the solution, while the appropriate amount of urea was weighed for each portion of the electrolyte. The pH value of the electrolyte was adjusted to 8.08 with 0.1 M NaOH. The pH measurements were carried out using a Denver model 20 pH meter with combination electrode (Denver Instrument Company, CO, USA). The

electrode was calibrated with pH 4.00 (±0.01), 7.00 (±0.01), and 10.00 (±0.01) commercial buffers (Reagecon, Shannon, Ireland and Reagena, Toivala, Finland).

3. Results

3.1. Optimization of the BGE solution used in the analysis of carboxylic acids

In this study, 2,3-PyDC was chosen for monitoring the aliphatic organic acids by an indirect UV detection. The effective mobility of chromophore was similar to the organic aliphatic acids under analysis conditions. In addition, a high sensitivity and a stable baseline with low noise were achieved. The pH and additives and some physical parameters were modified to reach optimum resolution with fast analysis time.

The preliminary tests showed that the best suited pH value of the electrolyte solution is between 8.0 and 8.5. This pH allows the use of organic buffers such as TRIS (Tris(hydroxymethyl)aminomethane), TAPS (N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid), and tricine (N-[tris(hydroxymethyl)methyl]glycine), since their useful working ranges are at pH scale 7–9, 7.7–9.1, and 7.4–8.8, respectively [36]. The buffering capacity of an electrolyte limits the possible variations in migration of ions caused by changes in pH and thereby aids to obtaining results with better reproducibility [37]. A zwitterionic tricine was chosen from these three organic buffers, since it gave the most repeatable consecutive runs. The concentration was optimized to 65 mM and the pH was adjusted to 8.06 with sodium hydroxide. At lower pH, maleate and succinate migrate together and phosphate disturbs the separation of acetate. However, at a pH above 8.5, the migration of lactate slows down and it merges with a negative system peak which migrates after all anionic analytes. The optimized separation of two inorganic anions and twelve aliphatic and one heterocyclic carboxylic acid under optimized electrophoretic conditions is presented in Fig. 1. Closer examination of the electropherogram indicates that the mobility of the probe is close to that of tartrate and phosphate. This can be observed in peak profiles: analytes having a higher mobility than phosphate are slightly fronting while the analytes migrating after acetate have tailing peak profile [38].

Three negative peaks can be seen in the electropherogram, one before the signal of sulphate, the second before acetate and the third after lactate. The sources of the peaks are chloride in the barium salt, absorbed carbonate, and tricine. Tricine produces a negative system peak since it acts as a co-ion of 2,3-pyrazine dicarboxylic acid. The addition of co-ion should be avoided as is stated by Doble and Haddad [38]. It will hinder the analysis of very low mobility analyte anions and secondly, analyte ions can displace the co-ions rather than the probe, which may lead to reduced detection signals. However, this non-recommendable choice to prepare BGE solution worked excellently for our purposes.

The bivalent metal cation can form weak complexes with dicarboxylic acids. Alkaline earth metal cations such as Ca^{2+}, Mg^{2+}, or Ba^{2+} can be employed in complexation reactions to enhance separation selectivity between closely related dicarboxylic acids [39,40]. In this study, barium cation was used for the complexation. Barium chloride was added to the electrolyte solution at the concentration of 2 mM. It has a positive effect especially on to the resolution between acetate and glycolate, which are unresolved from each other without Ba^{2+}. Moreover, the addition of a bivalent metal cation to the electrolyte solution improves the selectivity and resolution between dicarboxylic acids with a four carbon backbone, such as fumaric, maleic, succinic, malic, and tartaric acids. Fumaric acid is a *trans*-isomer and maleic acid a *cis*-isomer of butenedioic acid [41]. Another finding was that Ba^{2+} interacts with carbonate. Therefore, it is supposed that under the experimental conditions barium forms chelates with carbonate. $BaCO_3$ has low water solubility [42], and carbonate concentrations below 150 mg L^{-1} are not detectable (unpublished data). In addition, the low solubility of $BaCO_3$ may produce precipitations on to capillary surface and thereby affect to the repeatability of analysis. Therefore, to improve the capillary performance, rinsing step with a 0.1 M sodium hydroxide was added in analysis method. It is a notable finding that also the chromophore 2,3-pyrazinedicarboxylic acid forms complexes with bivalent cations [43]. This ability has a positive effect when organic acids are to be determined, since BGE masks the metal impurities which otherwise may repel on complex formation with the analytes [44].

Traditionally, urea has been used as a denaturant in the field of proteomics [45]. In the applications of capillary gel electrophore-

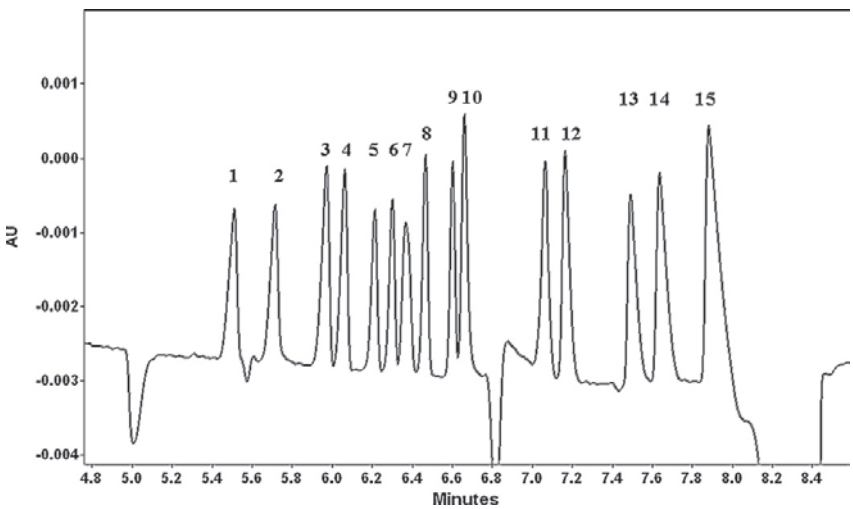

Fig. 1. Electropherogram of standard mixture of anionic analytes, 50 mg L^{-1} each. Peak numbering: 1, SO_4^{2-}; 2, oxalate; 3, formate; 4, malonate; 5, fumarate; 6, maleate; 7, succinate; 8, malate; 9, tartrate; 10, HPO_4^{2-}; 11, acetate; 12, glycolate; 13, citrate; 14, lactate, and 15, 2-furoate. Separation conditions: 250 V/cm; detection, 281 nm indirect mode; injection pressure, 0.5 psi for 10 s; capillary, 50/60 cm (L_{det}/L_{tot}), i.d. 50 μm; separation temperature 15 °C.

sis and capillary isoelectric focusing, urea is included in relatively high concentrations, up to 8 M, in the sample and/or run buffers to reduce conformer formation and prevent aggregation [45]. In our optimized BGE for carboxylic acids, 2 M urea was added to the electrolyte to increase the viscosity of the solution and even further improve the resolution between maleate, succinate, and malate. Polyethylene glycol, PEG 8000 at the concentrations of 0.01–0.1% (v/v), was also tested for enhancing the resolution between the comigrating acids, but a desirable effect was not obtained. During the analyses, traces of urea tend to crystallize on the vial caps, but this was mainly avoided by dipping the capillary ends in water immediately after the BGE rinse and by diminishing the running electrolyte volumes from 1.5 to 1.3 mL.

The detection wavelength for indirect UV detection was set to 281 nm, where the molar absorptivity of 2,3-PyDC was the highest. The developed electrolyte solution works well also for the monitoring of 2-furancarboxylic acid (2-furoic acid). This heterocyclic carboxylic acid is an oxidation product of dehydration of pentose sugars [11]. Typically, it has been analyzed with CE with some other aromatic carboxylic acids using direct UV detection [46]. The optimum wavelength for detecting 2-furancarboxylic acid with direct UV is 252 nm when the current BGE solution was employed. However, its signal is almost equal in sensitivity to the indirect UV detection. This finding allows us to use the developed BGE solution also for the analysis of 2-furoic acid with aliphatic acids.

Instrumental parameters were optimized in lesser extent than chemical parameters. However, the separation temperature and capillary length were tuned to appropriate values in order to maintain a fast separation and a high resolution. The effect of the separation temperature was studied in the range of 15–20 °C. The best suited separation temperature was 15 °C, where the quartet of fumarate, maleate, succinate, and malate were separated from each other. At the higher temperature the total migration time was slightly faster than at 15 °C, but the resolution of closely migrating analytes, i.e. between formate and malonate and also between tartrate and phosphate was poorer. During the method optimization, the total capillary length varied from 60 to 80 cm. The comparison of shorter and longer capillary revealed, that lengthening of the capillary does not improve remarkably the selectivity or resolution; it only unnecessarily slowed total analysis time. Thus, the optimized physical parameters in regards of fast separation and an appropriate resolution include the separation temperature of 15 °C and the total capillary length of 60 cm.

To demonstrate the applicability of the developed analysis method for the determination of selected anionic compounds, two red wine samples of various origins and two series of lignin processing samples were chosen.

3.2. Validation of the method

Linearity, repeatabilities, and detection limits were determined for each analyte. The calibration was made for 13 carboxylic acids and two inorganic anions. The percentage relative standard deviations for migration time and peak area were calculated for each analyte from the absolute values of intra- and interday runs (4 days, four repetitions/concentration level). The average of RSD% was calculated using the results of all calibration levels. The results are compiled in Table 1. All the calibrations were linear over the range announced, which can be seen from the coefficients of the regression lines. The concentration varied between analytes ranging from 2 to 75 mg L^{-1} of malonate and succinate to 2–150 mg L^{-1} of formate, acetate, and glycolate. In the studied concentration scales of analytes, the linearity of the calibration lines were good while the correlation coefficients ranged from 0.99 to 0.9994. The results show excellent reproducibility of the absolute migration times, and acceptable reproducibility of the absolute peak areas for all the analytes. The relative standard deviations (RSDs) of intraday results of peak areas varied from 4.3% for glycolic acid to 8.1% for lactic acid while the same values of interday results settled down to 5.3% for malonate and 8.8% for oxalate. The limits of detection (LOD) and quantitation (LOQ) were 1 and 2 mg L^{-1}, respectively, for all the analytes when injection of 0.5 psi for 10 min was employed. These concentrations are equivalent with approximately 60 and 150 fmol of injected amount per acid. The alkali rinse before analysis has a considerable effect on the reproducibility of absolute migration times: the RSD% varied from 0.2% to 1.1%. Without rinsing, the RSD values varied from 1.8 for oxalate to 5.6% for lactate indicating that the migration of last eluting analytes becomes slower due the adsorbed residues.

Addition of the sodium hydroxide rinsing step with stabilizes the migration time variation. This indicates that some compounds, analytes, or complexation products tend to attach to the capillary wall and are effectively removed by using a harsh rinsing step.

Table 1
Repeatability of the CZE analytical procedure: regression equations of standard mixtures, coefficient, and the average relative standard deviations (RSD%) of migration time and peak area for intra- and interday results.

Analytes in migration order	Peak area[a] (regression)			Average RSD% of standards, intraday (n = 4/concentration level)		Average RSD% of standards, interday (n = 4/concentration level, 3 days)	
	Equation ($y = ax + b$)	Coefficient (r^2)	Range (mg L^{-1})	Migration time	Area	Migration time	Area
SO_4^{2-}	$y = 118.12x - 94.299$	0.9983	2–100	1.0	6.6	1.2	7.8
Oxalate	$y = 117.41x - 83.865$	0.9986	2–100	0.2	6.0	0.6	8.8
Formate	$y = 159.16x - 72.137$	0.9994	2–150	0.2	5.2	0.6	5.7
Malonate	$y = 133.19x - 111.37$	0.9967	2–75	0.2	4.8	0.6	5.3
Fumarate	$y = 120.35x - 148.74$	0.9986	2–100	0.2	5.4	0.6	6.9
Maleate	$y = 93.841x - 92.285$	0.9988	2–100	0.2	6.0	0.6	6.2
Succinate	$y = 131.71x - 71.576$	0.9963	2–75	0.3	6.8	0.6	6.7
Malate	$y = 119.04x - 107.69$	0.9978	2–100	0.2	4.9	0.6	5.5
Tartrate	$y = 111.09x - 127.65$	0.9985	2–100	0.3	4.4	0.6	6.3
HPO_4^{2-}	$y = 126.26x + 52.746$	0.995	2–100	0.3	5.3	0.7	7.0
Acetate	$y = 171.6x - 287.45$	0.9953	2–150	0.3	6.4	0.6	6.2
Glycolate	$y = 192.36x - 503.56$	0.995	2–150	0.3	4.3	0.7	5.3
Citrate	$y = 148.71x - 203.3$	0.9962	2–100	0.3	4.8	0.7	7.9
Lactate	$y = 219.77x - 49.306$	0.9982	2–100	0.4	8.1	0.8	8.1
2-Furoate	$y = 454.29x - 32.011$	0.99	2–100	0.4	5.8	0.5	5.6

[a] Eight concentration levels of standards, four injections/concentration level.

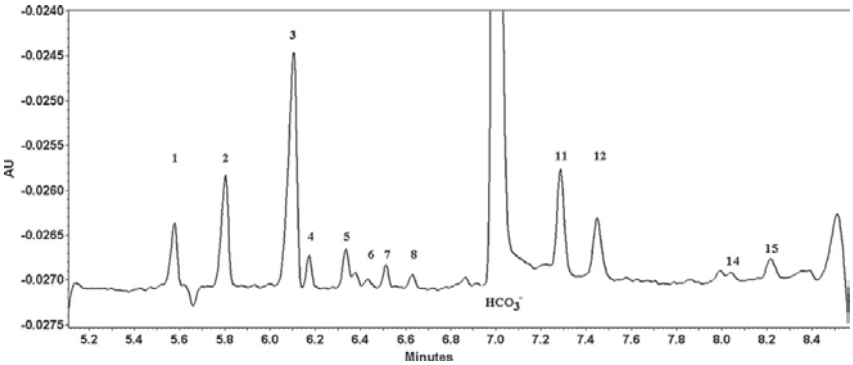

Fig. 2. Lignin degradation sample. The electropherogram is a typical example of lignin samples, only concentrations varied between the samples. Peak identification as in Fig. 1. The bicarbonate mainly originates from the alkaline treatment.

3.3. Analysis of real samples

3.3.1. Quantitative results of lignin samples

Softwood (SW) and hardwood (HW) kraft lignin samples were oxidized using a temperature of 90 °C and at 5 bar initial oxygen pressure. The carboxylic acid content of lignin degradation samples was measured at four points during the total 4 h reaction time. Fig. 2 shows a typical electropherogram of lignin samples. The detected carboxylic acids are both volatile and nonvolatile in nature. The previous group includes formic and acetic acids while the latter one contains dicarboxylic acids and hydroxy acids. Signals of oxalate, formate, acetate, and glycolate together with bicarbonate dominate the profile of the electropherogram. The pH of the oxidized samples is above 9. In those solutions carbon dioxide is solubilized to bicarbonate or carbonate. In Section 3.1 it was noted that carbonate concentrations below 150 mg L^{-1} are not detectable due to their complex formation with barium cation. The concentration of HCO_3^- is well above this limit; therefore its signal is visible. The main source of carbonate is sodium hydroxide which absorbs carbon dioxide. Malonate, fumarate, succinate, malate, and lactate can be found in smaller quantities. 2-Furancarboxylic acid has also been quantified in trace amounts, more in softwood samples than in hardwood samples. The amounts of 2-furoic acid found in the samples are at lower concentration levels than found in bleached effluents [27]. Some unidentified signals, next to fumarate and lactate, can be seen in the electropherogram. The results from the calculated concentrations of identified anions are compiled in Table 2. The development of concentrations of four major acids in respect to reaction time is presented in Fig. 3. Generally, the amount of individual acid increased during the first 3 h of reaction time and the evolution decreased during the last hour of oxygen treatment. These results lead to the assumption that under the presented oxidizing conditions the degradation of lignin will stabilize in 180 min, and only minor changes occur after this.

In the case of lignocellulose sample, it is assumed that the low molecular acids such as acetic acid and glycolic are degradation compounds of carbohydrates and only minority of acids come from the disruption of lignin [25,27]. However, the origin of the acidic products is difficult to interpret and the present experimental set-up enables unambiguous differentiation of the lignin derived products.

3.3.2. Quantitative results of wine samples

Two wine samples from different origins were used as the model samples of acidic matrix. The common denominator for these samples is Pinot noir grape and production year. Aliphatic carboxylic acids were analyzed from these samples after 1:20 (v/v) dilution with Milli-Q water. Fig. 4 shows a typical electropherogram obtained from the wine samples. Peak identification was performed by standard addition and by comparing the migration times with those obtained with a standard solution.

The major acids found in samples were succinic, tartaric, acetic, and lactic acids. Malic and citric acids have been found in minor quantities. Two inorganic anions, namely sulphate and phosphate, were found. Similar acid contents have been presented by Fung and Lau [5] and Tanyanyiwa et al. [47]. The compiled results of wine samples are presented in Table 3.

As can be seen, there were noticeable variations in acid concentrations between the wines. This may be an indication of the differences in fermentation conditions. More citric, malic, succinic, and tartaric acids were found in the Patagonian product, while the amount of lactic acid was larger in the Swiss wine. Both wines contained sulphate and phosphate.

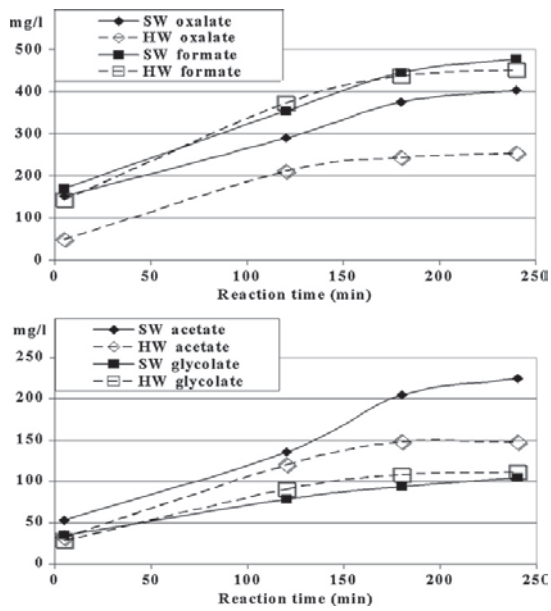

Fig. 3. The change of concentrations of four major anions in softwood and hardwood samples in relation to reaction time.

Table 2
Quantitative results of lignin samples after different processing times at oxidation conditions of 5 bar and 90 °C.

Sample	Time (min)	Anion (mg L^{-1})										
		SO_4^{2-}	Oxalate	Formiate	Malonatec	Fumarate	Succinate	Malate	Acetate	Glycolate	Lactate	2-Furoate
Softwood												
SW 5	5	77	49	143	<LOQ	ND	<LOQ	ND	32.4	28.2	<LOQ	ND
SW 120	120	98	211	372	29.9	49.6	26.7	15.8	120	90.5	ND	21.2
SW 180	180	99	243	436	36.6	69.2	29.7	15.9	145	108	ND	<LOQ
SW 240	240	104	254	451	37.5	70.9	27.9	15.7	148	112	<LOD	<LOQ
Hardwood												
HW 5	5	129	152	170	34.1	22.1	ND	ND	53.0	35.0	ND	ND
HW 120	120	152	290	355	36.3	84.0	20.5	<LOQ	136	78.8	ND	ND
HW 180	180	179	375	445	43.6	97.8	<LOQ	<LOQ	205	93.8	ND	ND
HW 240	240	19 1	402	477	45.0	101	<LOQ	<LOQ	225	105	<LOD	<LOQ

ND: not detected, LOD = 1 mg L^{-1}, LOQ = 2 mg L^{-1}.

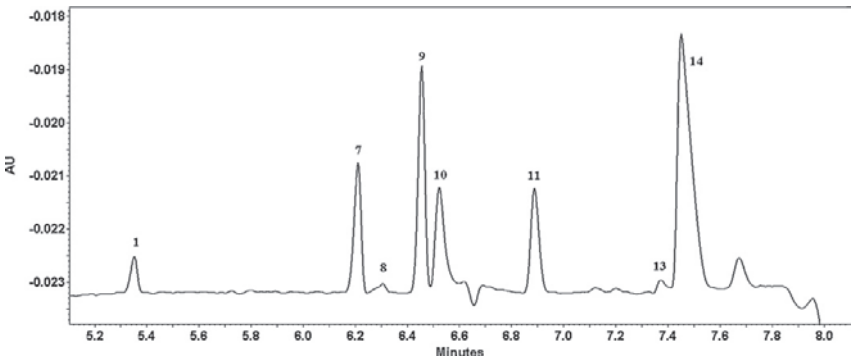

Fig. 4. Wine sample "Patagonia", diluted to 1:20 (v/v). The electropherogram is typical to wines, only concentrations varied. Peak identification as in Fig. 1.

Table 3
Quantitative results of wine samples.

Analyte	Wine Patagonia (mg L^{-1})	±RSD%	Wine Switzerland (mg L^{-1})	±RSD%
HPO_4^{2-}	843	3.4	687	3
SO_4^{2-}	268	4.3	229	1.6
Acetate	529	2.8	611	1.9
Citrate	82	12.9	ND[a]	–
Lactate	1602	2.7	3418	1.4
Malate	79	2.1	ND	–
Succinate	830	3.1	341	8.1
Tartarate	1528	3.4	1092	5

[a] ND: not detected.

Tartaric, citric, and malic acids come from the grape, whereas lactic, succinic, and acetic acids originate from the fermentation process [48]. Lactic acid is a major organic acid in wine and it is thought to have a great influence on the taste [2]. In addition, tartaric acid gives a sharp and hard taste to wine therefore high quality wines contain less of this acid. For example, botrytised wines contain more than 2 g L^{-1} tartaric acid [32]. The quantification of acids allows controlling the acidity during the different steps of the winemaking process, such as alcoholic fermentation, malolactic fermentation, and aging [48]. The source of sulphate may be the sulphite which is added to wines to prevent oxidation and to stabilize the chemical composition. Phosphate concentration reflects the amount of soil phosphates. Its concentration depends on the amount and type of fertilizers used on the vineyard [49].

4. Concluding remarks

A simple, fast, and repeatable method was developed for the analysis of low molecular weight carboxylic acids. The BGE has a significant effect on the separation efficiency of analytes. The addition of a complex forming cation to the BGE solution clearly enhances the selectivity of closely related acids. Moreover, by including urea in the electrolyte, it is possible to even further improve the resolution. The buffering of the electrolyte with the zwitterionic tricine decreases the separation current and thus reduces the Joule heating. In addition, the selected detection wavelength allows the monitoring of the heterocyclic carboxylic acid together with aliphatic ones with great sensitivity. The method is linear over the range from 2 to 150 mg L^{-1}. The optimized method is suitable for reliable analysis of carboxylic acids in various samples. Only minimal sample preparation is needed prior to analyses. The presented method was applied to the determination of low molecular weight carboxylic acid contents of two different types of samples where the acid profile has great importance. Wine is an example of food samples while the lignin degradation samples represent a sample type found in pulp and paper related research. Both sample types are originally natural products which are subjected to various biochemical or chemical processes. Therefore it is not surprising that the same carboxylic acids can be found in different concentration ratios in both sample groups. The ability of simultaneous monitoring 2-furancarboxylic acid together with aliphatic acids is beneficial in the analysis of major anions in lignin degradation processes.

Acknowledgments

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PAPER V

Catalysed alkaline oxidation as a wood fractionation technique

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CATALYSED ALKALINE OXIDATION AS A WOOD FRACTIONATION TECHNIQUE

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Alkaline oxidation (AlkOx) is an effective fractionation technique for lignocellulosic raw materials. The efficiency of the AlkOx treatment can further be enhanced by using a catalyst (CatOx). Both CatOx and AlkOx provide a fiber fraction containing readily hydrolysable carbohydrates that can be utilized in biotechnical processes and a liquid fraction containing solubilized lignin and reaction products from various biomass components. The effects of different fractionation conditions on yields and chemical composition of solubilized and insoluble fractions were investigated. Two temperatures and two reaction times were studied with and without a catalyst. The composition and content of carbohydrates in the fiber and liquid fractions were examined. The generation of aliphatic carboxylic acids as oxidation products was also investigated. The catalytically assisted oxidation was more efficient than the alkaline counterpart in dissolution of wood components under a four-hour treatment period resulting in higher dissolution of hemicelluloses. A longer reaction time of 20 hours leveled out the differences between the oxidation processes. Comparison of different bases showed that similar solubilisation of dry matter was obtained with NaOH, KOH, and Na_2CO_3. Oxidation in Na_2CO_3 caused higher dissolution of glucomannan and greater acid production. The dissolution of hemicellulose and lignin, and their oxidation to acids was most efficient in the first 4 hours of oxidation.

Keywords: Catalysis; Alkaline oxidation; Chromatography; Capillary electrophoresis; Spruce; Pretreatment; Fractionation

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INTRODUCTION

The use of lignocellulosic biomass as a source of chemicals and fuels has been a subject of interest recently due to concerns about climate change and the need to find an alternative to fossil resources. Currently, the main transport biofuel products on the market are bioethanol, biodiesel, and biogas. Bioethanol has the largest market by far, although biodiesels are also expected to increase in popularity (Mosier et al. 2002). Biomass valorization concentrates mainly on cellulose, but an economically viable production necessitates the valorization of all components of lignocellulosic biomass. Approximately only 2% of the lignins available from the pulp and paper industry are used commercially, and the remainder is burned as a low value fuel: however, lignin has significant potential as an aromatic resource for bulk chemical and fuel production (Zakzeski et al. 2010; Hallac et al. 2009).

Pretreatment is one of the most critical steps in converting lignocellulosic materials into bioethanol or other chemicals produced by fermentation. It decomposes the cell wall and separates the material into its components, partly degrading the biopolymers into smaller fragments. This kind of fractionation is needed to produce carbohydrate material readily hydrolysable to sugars by enzymes (Chen et al. 2006). Efficient pretreatment also reduces the cellulose crystallinity and increases the porosity of the material; however, fractionation should not cause remarkable carbohydrate yield losses (Sun and Cheng 2002). In addition, production of toxic or inhibiting compounds should be minimal, and the pretreatment should be technically and economically feasible (Kumar et al. 2009).

Several pretreatments have been studied relative to degradation purposes (Zheng et al. 2009). Among these are steam explosion, organosolv pulping, acidic pretreatments, and wet oxidation

Uncatalyzed steam-explosion, also named as autohydrolysis, is a technology in which only steam water is used to destroy the physical structure of lignocellulosic material. During the pretreatment, the hemicellulose is often hydrolyzed by organic acids such as acetic acids and other acids formed from acetyl or other functional groups, released from biomass. In addition, water itself also possesses certain acidic properties at high temperature, which further catalyze hemicellulose hydrolysis (Avellar and Glasser 1998; Taherzadeh and Karimi 2008). Steam-explosion is typically conducted at a temperature of 160 to 270℃ for several seconds to a few minutes before pretreated contents are discharged into a vessel for cooling. The action of steam explosion can be further enhanced by addition of catalyst such as SO_2, H_2SO_4, and CO_2. Catalyzation improves hemicellulose removal and generates less inhibiting compounds (Zheng et al. 2009).

In the organosolv pretreatment process, organic or aqueous organic solvent with inorganic acid catalysts are used to dissolve the lignin and separate the fibres (Sun and Cheng 2002). Organosolv pulping can remove lignin from fibres selectively, resulting in a pulp of higher yield and viscosity and less condensed residual lignin (Lohrasebi and Paszner 2001). The benefit of the organosolv process is the possibility of recycling the cooking liquid, which decreases the process costs. In addition, it is also obligatory, since the solvent may be may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation (Sun and Cheng 2002).

Dilute acid pretreatment is also a widely used pretreatment method. It is conducted typically using sulfuric acid at high temperature (160 to 200 °C) (Shuai et al 2010). Dilute acid treatment dissolves hemicellulose and partially hydrolyzes cellulose. Serious equipment corrosion problems and extensive condensation of lignin limit the usefulness of this pretreatment. In addition, dilute acid pretreatment can achieve satisfactory levels of cellulose saccharification for agricultural residues and some hardwood species, but it is not effective for softwoods. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) has been recently developed for robust and efficient bioconversion of softwoods (Zhu et al. 2009). The process involves treatment of wood chips under acidic conditions using 8 to 10% bisulfite and 1.8 to 3.7% sulfuric acid at 180 °C for 30 min. By this pretreatment almost complete hemicellulose separation, partial delignification, and lignin sulfonation were achieved, offering improved recovery of fermentable sugar, when compared with dilute acid pretreatment. Moreover, a benefit of

SPORL pretreatment is its applicability of variety lignocellulosic raw material (Tian et al. 2011).

The above mentioned pretreatment methods are usually carried out under acidic conditions. Wet oxidation is a pretreatment that is performed under neutral environment. It involves the treatment of the biomass with water and air, or oxygen, at temperatures between 120 and 350°C and at elevated pressures (> 1 MPa) (McGinnis et al. 1983; Bjerre et al. 1996; Palonen et al. 2004).

In more recent developments, wet oxidation has been combined with an alkaline-aided hydrolysis, utilizing either sodium carbonate or lime as the base (Bjerre et al. 1996; Chang et al. 2001). The alkaline wet oxidation is typically applied to biomasses having a low lignin content, such as wheat straw, clover, and ryegrass (Galbe and Zacchi 2002; Bjerre et al. 1996; Martin et al. 2008). This process has also been used as a pretreatment of softwood and hardwood in order to produce bioethanol and biogas (Palonen et al. 2004). The alkaline wet oxidation process has been found to convert many organic polymers to oxidized compounds, such as low molecular weight carboxylic acids, or even to CO_2 and H_2O (Taylor and Weygandt 1974; Klinke et al. 2002); even 65% of wheat straw lignin could be removed by wet oxidation.

Enhancement of the alkaline oxidative fractionation by the addition of a catalyst is a new attractive approach (Korpi et al. 2004), especially in the case when pH of the reaction is strongly alkaline (Hakola et al. 2010). Alkaline oxidation can be catalyzed by a copper-phenanthroline complex, while copper(II) ions form oxygen-activating copper–diimine complexes e.g with 1,10-phenanthroline (phen) and its substituted derivatives (Korpi et al. 2006). According to Korpi et al. (2004) the in situ complexed Cu–phen is an active catalyst for the oxidation of lignin compounds and is thus a potential catalyst for the biomass pretreatment process.

Softwoods are generally considered to be a difficult lignocellulosic raw material to hydrolyze to sugars for fermentation, primarily owing to the nature and amount of lignin. When compared to hardwoods, softwoods contain more hemicellulose, and they have lower xylose content but higher mannose content (Mabee et al. 2006).

The goal of the study was to evaluate an alkaline pretreatment method for the production of bioethanol from softwood. The focus of the present paper is on the chemistry that takes place in alkaline oxidation and the effects of different process parameters, in particular the role of the catalyst in a laboratory scale batch reactor. Other process parameters studied were temperature, treatment time, reaction volume, liquid/gas ratio, and the alkali source.

The decomposition of spruce raw material was monitored by analyzing the carbohydrate composition and yield of the fiber fraction and the compounds formed during the fractionation and dissolved in alkaline liquors. Soluble carbohydrates were analysed with anion exchange chromatography, dissolved lignin with UV measurements, and the formation of small aliphatic carboxylic acids with capillary electrophoresis (CE). The evaluation of the effects of pretreatments on enzymatic hydrolysis yield will be reported separately.

EXPERIMENTAL

Raw Materials

Three kind of particle size were used, depending on the reactor volume. Industrial wood chips consisting mainly of Norway spruce (*Picea abies*) were obtained from a Finnish pulp mill. Part of the chips was ground into wood meal using a Wiley-mill equipped with a 5 mm sieve. Softwood saw dust was collected from a Finnish saw mill and had an average particle size of 2 mm x 2 mm x 10 mm.

Oxidation Treatments

Four different decomposition treatments were carried out. In the first and second series, catalyzed alkaline oxidation (CatOx) was compared with alkaline oxidation (AlkOx). Reaction volume, temperature, and time were variables in both series, as shown in Table 1. In addition, an excess of oxygen was fed into the reactor to kept pressure at a constant level in series I. Sodium carbonate was the alkali used in these experiments. In the third series, oxidations using different alkali sources (calcium hydroxide, sodium hydroxide, potassium hydroxide, and sodium carbonate) without a catalyst were compared. Detailed reaction conditions are listed in Table 1. The fourth series was also catalytically assisted, but the reaction volume was one liter and the experiment was performed in four parallel reactions with different reaction times (1, 4, 8, and 20 h).

In the case of catalyzed oxidation, 0.84 mmol L^{-1} of $CuSO_4*5H_2O$ and 1.66 mmol of L^{-1} phenanthroline were added to the reaction solution. The reagents were dissolved and added to the preheated autoclave (volume of 1 or 2 liters) equipped with a stirrer or added to a stirred tank reactor (volume of 40 liters). The substrate was added to the reactor, stirring was started, and the autoclave was closed and pressurized immediately with 10 bar of oxygen and then heated to the reaction temperature of either 120 °C or 140 °C. The liquid to wood ratio and oxygen loading as well as consistency are compiled in Table 1.

In all experimental series, the reactors were cooled down after the reaction completion and the pressure was released. At temperatures below 50 °C, the reactor was opened and the solid material was washed and separated with vacuum filtration to remove any soluble material. The sugar compositions of the filtrates were then analysed. Part of the filtrate was acidified with 1 M HCl to a pH of 2.5. The precipitate (lignin and some carboxylic acids) was recovered by centrifugation (4000 rpm, 15 min), washed with water (adjusted to a pH of 2.5), centrifuged, and finally freeze-dried.

Analytical Methods

Carbohydrates

The carbohydrate content and composition were determined as monosaccharides using acid hydrolysis and high performance anion exchange chromatography with pulse amperometric detection (HPAEC/PAD) (Willför et al. 2009). A CarboPac PA-1 column 4×250mm (Dionex, Sunnyvale, CA, USA) was coupled with a Dionex ICS-3000 series chromatograph equipped with a pulsed amperometric detector (Dionex ICS-3000). For monosaccharide analysis the system was equilibrated with 15 mM sodium hydroxide.

Table 1. Conditions Used in the Decomposition Tests

Series	Sample code used in Figures and Tables	Sample form	Liquid–to–wood ratio (v/w)	Alkali (conc. 0.25 M)	Catalyst	T (°C)	Oxygen loading (g O_2/g wood)	Liquid volume / reactor volume (Liter) (% fill)	Reaction time (hours)
I	AlkOx 120 °C 4 h 75 %	Chips	20	Na_2CO_3	No	120	0.1(*	30 / 40 (75 %)	4
	CatOx 120 °C 4 h 75 %	Chips	20	Na_2CO_3	Yes	120	0.1(*	30 / 40 (75 %)	4
	AlkOx 140 °C 4 h 25 %	Chips	20	Na_2CO_3	No	140	0.7(*	10 / 40 (25 %)	4
	CatOx 140 °C 4 h 25 %	Chips	20	Na_2CO_3	Yes	140	0.7(*	10 / 40 (25 %)	4
II	CatOx 120 °C 20 h 50 %	Wood meal	20	Na_2CO_3	Yes	120	0.2	1 / 2 (50 %)	20
	CatOx 120 °C 20 h 10 %	Wood meal	20	Na_2CO_3	Yes	120	2.1	0.2 / 2 (10 %)	20
	AlkOx 120 °C 20 h 50 %	Wood meal	20	Na_2CO_3	No	120	0.2	1 / 2 (50 %)	20
	AlkOx 120 °C 20 h 10 %	Wood meal	19	Na_2CO_3	No	120	2.1	0.2 / 2 (10 %)	20
III	AlkOx $Ca(OH)_2$ 120 °C 20 h 10 %	Saw dust	20	$Ca(OH)_2$	No	120	2.1	0.1 / 1 (10 %)	20
	AlkOx NaOH 120 °C 20 h 10 %	Saw dust	20	NaOH	No	120	2.1	0.1 / 1 (10 %)	20
	AlkOx KOH 120 °C 20 h 10 %	Saw dust	20	KOH	No	120	2.1	0.1 / 1 (10 %)	20
	AlkOx Na_2CO_3 120 °C 20 h 10 %	Saw dust	20	Na_2CO_3	No	120	2.1	0.1 / 1 (10 %)	20
IV 1-4		Saw dust	20	Na_2CO_3	Yes	120	2.1	1 / 2 (50 %)	1, 4, 8 or 20

* oxygen loading at start; pressure was kept constant by feeding excess O_2.

After sample injection, 15 mM sodium hydroxide flowed through the column for 2 min, and from 2 to 36 min 100% of ultra-pure water was run isocratically. A solution of 300 mM NaOH was added to the column effluent before the PAD cell at a flow rate of 0.4 mL min^{-1}. The column was washed with a solution of 100 mM NaOH and 300 mM sodium acetate for 3 min and then washed with 300 mM sodium hydroxide for 4 min. The flow rate was set to 1 mL min^{-1}, column temperature to 30°C, and sample temperature to 15°C. Injection volume was 25 µL. The resulting data were processed using Chromeleon software.

Carboxylic acids

Separations of the carboxylic acid standards and liquid fractions of the oxidation samples were performed using a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a photodiode array UV detector. The background electrolyte (BGE) was fine tuned to enhance the resolution between closely migrating acids and for quantifying the C(5)-C(6) hydroxy acids (Rovio et al. 2010). Briefly, anionic compounds were analyzed using the BGE solution containing 20 mM 2,3-pyrazine dicarboxylic acid, 30 mM tricine, 2 mM $BaCl_2$, 0.5 mM cetyltrimethyl-ammonium bromide, and 2 M urea at a pH of 8.06 (± 0.02, adjusted with triethanol-amine). Uncoated fused silica capillaries with a 50 µm I.D. and a length of 100/110 cm (effective length/total length) were employed in the analyses. The samples were injected at a pressure of 0.5 psi (34.5 mbar), and the injection time was set to 20 s. The capillary and samples were thermostatted to +15 °C. The detection wavelength was set to 281 nm using an indirect detection mode. Before the measurements, the new capillaries (from Teknolab Trollåsen, Norway) were conditioned by sequentially rinsing with 0.1 M sodium hydroxide, 0.1 M HCl, and ultra-pure water. The capillaries were rinsed with each solution for 20 min and then with an electrolyte solution for 5 min. Between analyses, the capillaries were rinsed with 0.1 M HCl for 3 minutes, 0.1 M NaOH for 1 min, and the electrolyte solution for 5 min, all under a pressure of 20 psi. Prior to CE analyses, the oxidation samples were diluted to a ratio of 1:10 (v/v) with ultra-pure water.

Lignin content

The content of the dissolved, aromatic lignin was measured from the soluble fraction UV spectroscopy at 280 nm using an absorptivity of 20 L g^{-1} cm^{-1} (Tamminen and Hortling 2001).

Total organic carbon

Total organic carbon (TOC, SFSEN 1484:1997) was determined at Kymen Laboratorio Oy (Finland).

Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) was performed in a Waters HPLC system. Two Ultrahydrogel (pore sizes of 250 Å and 120 Å, Waters Assoc. USA) columns were linked in series. The detection was carried out with an UV at 280 nm and refractive index (RI) detectors in series. Isocratic chromatography was performed using 0.1 M $NaNO_3$ as the eluent, which was pumped through the columns at a rate of 0.5 mL/min. Each sample

was diluted with a ratio of 1:10 (v/v) with eluent and filtered with 0.45 um disposable filters prior to the injection of 50 µL into the SEC system. The analysis temperature was 30 °C. The molecular weight distributions (MWD) and the average number and weight average molecular weights (Mn, Mw) of the lignin and polysaccharides were calculated using pullulan standards (5900-212000 Da). The system was controlled and data were analysed using Empower 2 software.

RESULTS AND DISCUSSION

General Composition of the Fractions

Catalyzed alkaline oxidation produces fiber fractions with high enzymatic hydrolysability (Hakola et al. 2010). The outcome of both the alkaline and catalyzed reactions can be compared by examining the mass loss, i.e. the proportion of the dissolved lignin and hemicellulose from the original dry weight of the wood.

During the decomposition of wood under oxygen-alkaline conditions, with or without a catalyst, the wood material was divided into two fractions: the solid cellulose rich fraction and the water soluble fraction. Figures 1a, 1c, and 1e present the percentage portions of both the solid and soluble fractions obtained using different reaction conditions. A more detailed distribution of the monosaccharides, small aliphatic carboxylic acids, and lignin in the soluble fraction is depicted in Figs. 1b, 1d, and 1f.

Catalytically assisted decomposition of wood under oxygen-alkali conditions increased the degradation of spruce compared to its counterparts treated in alkali without catalyst (Figs. 1a and 1c). The resulting material from batch reactor was, however, inhomogeneous, containing also unreacted particles, and further scale-up of the technique is needed in order to evaluate its feasibility in full scale industrial process.

A clear difference between non-catalyzed and catalyzed treatment can be observed in the 120°C experiment, where the portions of soluble fractions were 14% and 28%, respectively. By increasing the reaction temperature to 140°C and lowering the filling ratio of the reactor, the difference became smaller, but still the catalyzed reaction was more efficient. Increasing the reaction time to 20 h leveled out the dissolution efficiency between the AlkOx and CatOx treatments, while the proportions of fiber fraction and dissolved fraction became similar (Fig. 1c). The loading of the reactor had a small effect on fractionation. The lower filling ratio, 10% versus 50%, enhanced the dissolution, however.

Over 35% of the dry matter was solubilized in the alkaline or catalytic oxidation when the loading was 10% or 25% of the reactor volume (Figs. 1a and 1c). The higher oxygen volume as a result of lower liquid volume likely enhanced the oxidation reaction and solubilisation. Lower liquid volumes might improve the mixing of the suspension, decrease the concentration gradients, and increase the solubilisation of oxygen throughout the reaction. These observations need to be taken into account when scaling up the method. The proportions of monosaccharide, carboxylic acid, and lignin in the soluble fractions followed the trend of the total soluble fraction (Fig. 1b). In the four-hour experiments, the proportions of chemical components in the liquid fraction were 6 to 12%, 10 to 20%, and 1 to 4% of the raw material dry weight for lignin, carboxylic acid,

and monosaccharides, respectively. The increase in the reaction time from 4 h to 20 h had greater affect on the proportion of dissolved lignin and monosaccharides, while the proportions nearly doubled when compared to the shorter treatment (Fig. 1d). Catalytically assisted treatment produced only more dissolved monosaccharides than the alkaline treatment, but its effect on lignin solubility was not as clear. Most likely, the dissolution of lignin was more affected by the increased reaction time coupled with the action of alkali.

Quite similar solubilisation was obtained with the three bases with the exception of $Ca(OH)_2$ (Fig. 1e). Among these three experimental series the highest yield of dissolved substances was 43%, which was achieved with 0.25 M Na_2CO_3. The cumulitive amount of the recognized dissolved compounds was about 30% (Fig. 1f), which means that 3 to 11% of the dissolved matter remained unidentified. The unexplained part may include other degradation products that cannot be determined with the applied methods, and unidentified carboxylic acids. In addition, among the uncalculated compounds is carbon dioxide, which is the final degradation product of carboxylic acids (Suzuki et al. 2006).

The fractionation with calcium hydroxide was more complicated to study, since calcium hydroxide was not soluble in the applied concentration and conditions. This also explains the high yields of fiber fraction in the experiments (Fig. 1e). The unrealistic high result can be explained with undissolved $Ca(OH)_2$, oxalic acid which precipitates as calcium oxalate, and undissolved lignin, which was not monitored among the identified compounds in the dissolved fraction (Fig. 1f). The differences in chemical compositions of the dissolved fractions of NaOH, KOH, and Na_2CO_3 in 20 hours of treatment were minimal.

Carbohydrates

The carbohydrate content of the spruce raw material was 66 and 65% for the chips and saw dust, respectively. A minor part (1 to 6% of the raw material dry weight) of the carbohydrates in the raw material was found in the soluble fractions, whereas most of the carbohydrates remained in the solid fraction after the fractionation (Fig. 2). The carbohydrate content of the solid fraction after fractionation was between 40 and 60% of the raw material dry weight, and the respective carbohydrate yield was between 66 and 92% of the raw material carbohydrates. The solubilized fraction contained only oligosaccharides and no monosaccharides. Most likely, the reducing ends of the soluble carbohydrates were oxidized under experimental conditions, as the soluble fraction gave no response to reducing sugars assay (data not shown). In general, the absence of monosaccharides and the low concentration of solubilized oligosaccharides may be explained by their further oxidation into carboxylic acids. As the total carbohydrate yield varied between 72% and 99% of the raw material carbohydrate content, it is obvious that carbohydrate yield losses occurred.

The composition of the dissolved carbohydrate material is basically similar to that reported earlier for wet oxidized spruce (Palonen et al. 2004), i.e. xylose, mannose, arabinose, galactose, and glucose, illustrating that mainly the hemicelluloses (arabinoxylan and galactoglucomannan) dissolve while the cellulose remains in the fiber fraction (Fig. 2).

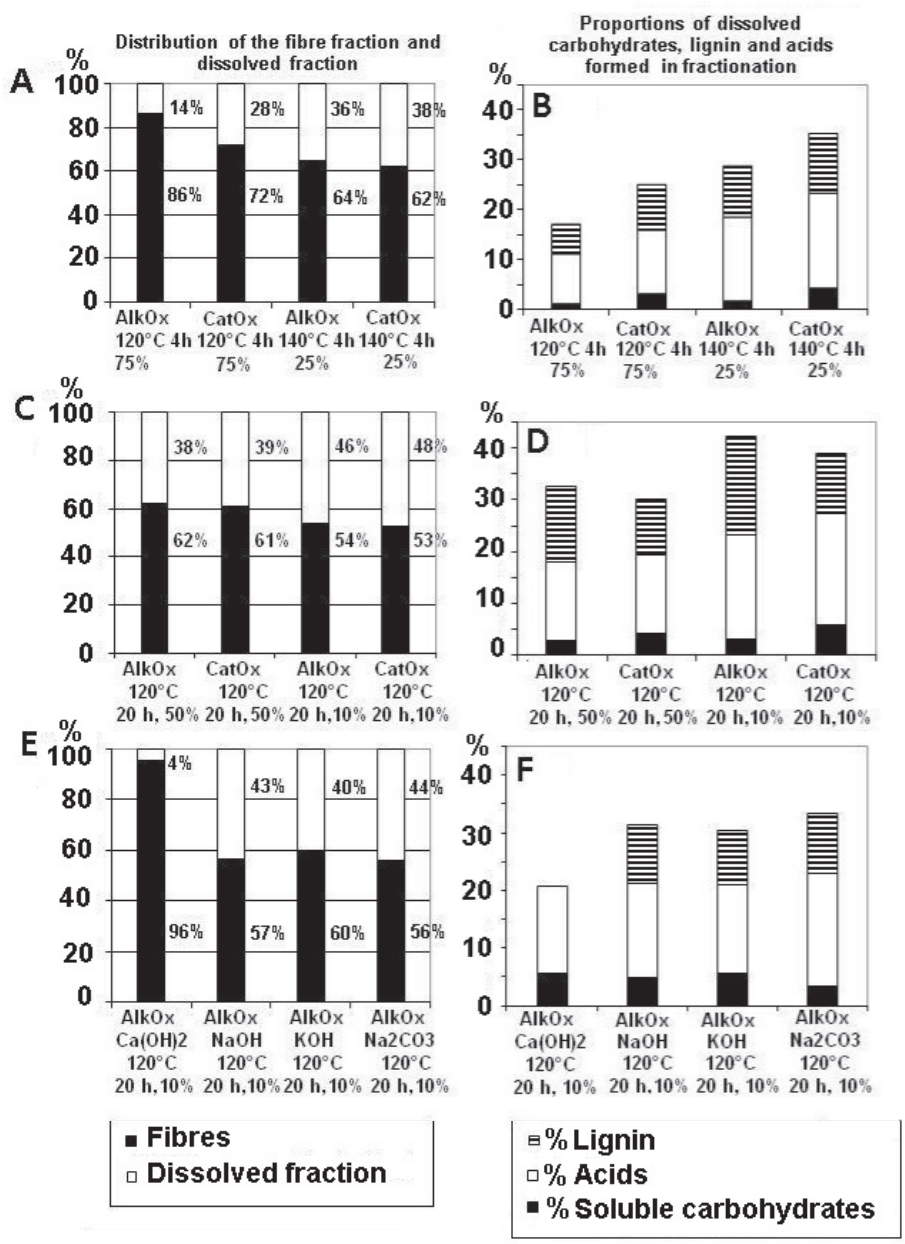

Fig. 1. Percent distribution of fibre fraction and soluble fraction after oxidation experiments in series I (A), II (C), and III (E). Portions of soluble carbohydrates, lignin, and small carboxylic acids found in dissolved fraction in series I (B), II (D), and III (F). The details of fractionation conditions of the samples are described in Table 1.

In addition, the figures show that under more severe conditions, i.e. with a catalyst, 20 hours of reaction time, and a higher temperature of 140 °C, there was a higher carbohydrate content in the liquid fraction. The shorter reaction time (4 h), lower temperature (120 °C), and Na_2CO_3 as an alkali all resulted in lower carbohydrate dissolution.

The oxidations were carried out with either 0.25 M Na_2CO_2, NaOH, KOH, or $Ca(OH)_2$, producing highly alkaline conditions (pH≥12) early in the reaction. During oxidation part of the alkali was consumed and after 20 hours the pH was usually between 7.5 and 8.5.

The dissolution of arabinoxylan was higher than that of galactoglucomannan in all experiments, but an especially clear difference was seen between the CatOx and AlkOx treatments (Figs. 2a and 2c). In addition, the comparison of alkali sources showed that the dissolution of galactoglucomannan was enhanced when $Ca(OH)_2$, KOH, or NaOH was used. The lower mannose content after oxidation with Na_2CO_3, however, can be explained by the degradation of galactoglucomannan, derived monosaccharides, and more pronounced acid production (Figs. 1f and 2e). The monosaccharide content and composition of the fiber fraction were also determined (Figs 2b, d, f). As expected, the majority of this fraction consisted of cellulose, but portions of 17-22%, 14-19%, and 12-18% of hemicelluloses, galactoglucomannan and arabinoxylan, were also observed in series I-III, respectively.

Table 2 compiles the results of the carbohydrate yields in the solid and liquid fractions. The total monosaccharides yield reveals clearly how much carbohydrate was lost, i.e. it gives information about the magnitude of the degradation of carbohydrates in the acids. This range varied between 3 and 28 percent, with the least degradation occurring with AlkOx, at 120°C, for 20 h, and a filling ratio of 50%, and the most degradation occurring with AlkOx, Na_2CO_3, at 120°C, for 20 h, and a filling ratio of 10%, indicating that the high filling ratio of the reactor hindered the oxidation reactions.

Lignin

Alkaline SEC is a common relative method for determining the molecular weight of lignins, as reviewed by Baumberger et al. (2007). The separation was calibrated here with pullulan standards in accordance with quoted literature, which can be considered as adequate for comparative purposes. SEC analyses of the dissolved fractions of series I and II reveal that the dissolved fraction contains both lignin and carbohydrate-based oligomeric substances having rather low molecular weights monitored with UV and RI detectors (Table 3). The four-hour reaction time with AlkOx treatment and a filling ratio of 75 % was not sufficient to dissolve lignin or hemicelluloses completely, and it was supposed that only small molecular weight lignin or oligosaccharides were dissolved; therefore, the molecular weight results obtained from this experiment are not comparable with its CatOx counterpart. The CatOx fractionation produced smaller lignin and polysaccharide oligomers than the AlkOx treatment during the 20 hour oxidation. This difference is not as clear as in the four-hour fractionations, except in the case of 140 °C. In addition, the polydispersities were lower in the CatOx samples than in the AlkOx samples in the 20 hour treatments. This indicates relatively narrow molecular weight distributions of dissolved substances.

Table 2. Carbohydrate Fraction Yield and Total Yield

SAMPLE	Fraction	GLU	MAN	XYL	GAL	ARA	Total mono-saccharides
AlkOx 120°C 4 h 75%	solid	103	59	86	47	77	92
	liquid	0.1	1	6	11	14	1
	total	103	60	92	58	90	93
CatOx 120°C 4 h 75%	solid	89	56	61	30	49	78
	liquid	0.7	6	20	21	30	4
	total	89	62	81	50	79	82
AlkOx 140°C 4 h 25%	solid	99	41	71	29	50	83
	liquid	0.2	1	12	14	26	2
	total	99	42	83	43	76	86
CatOx 140°C 4 h 25%	solid	100	48	54	20	38	83
	liquid	1.1	7	28	21	37	6
	total	101	55	82	42	74	89
CatOx 120°C 20 h 50%	solid	105	61	46	11	24	88
	liquid	0.9	11	40	37	52	8
	total	106	72	86	49	75	96
CatOx 120°C 20 h 10%	solid	106	55	41	6	19	87
	liquid	1.1	14	42	37	52	9
	total	107	69	83	43	71	96
AlkOx 120°C 20 h 50%	solid	109	61	64	14	37	93
	liquid	0.3	3	24	21	41	4
	total	110	64	88	36	78	97
AlkOx 120°C 20 h 10%	solid	102	49	57	8	30	85
	liquid	0.3	3	25	25	47	5
	total	103	52	82	33	78	90
AlkOx $Ca(OH)_2$ 120°C 20 h 10%	solid	93	26	31	5	20	70
	liquid	2.0	15	36	18	51	9
	total	95	40	66	23	71	78
AlkOx NaOH 120°C 20 h 10%	solid	84	35	36	12	24	67
	liquid	1.0	11	31	38	59	8
	total	85	46	67	50	83	74
AlkOx KOH 120°C 20 h 10%	solid	88	41	36	40	26	70
	liquid	1.4	15	31	40	53	15
	total	89	56	68	79	78	85
AlkOx Na_2CO_3 120°C 20 h 10%	solid	83	36	40	11	27	66
	liquid	0.5	5	25	26	56	5
	total	84	41	66	37	83	72

* Yield is expressed as % of the raw material carbohydrate content

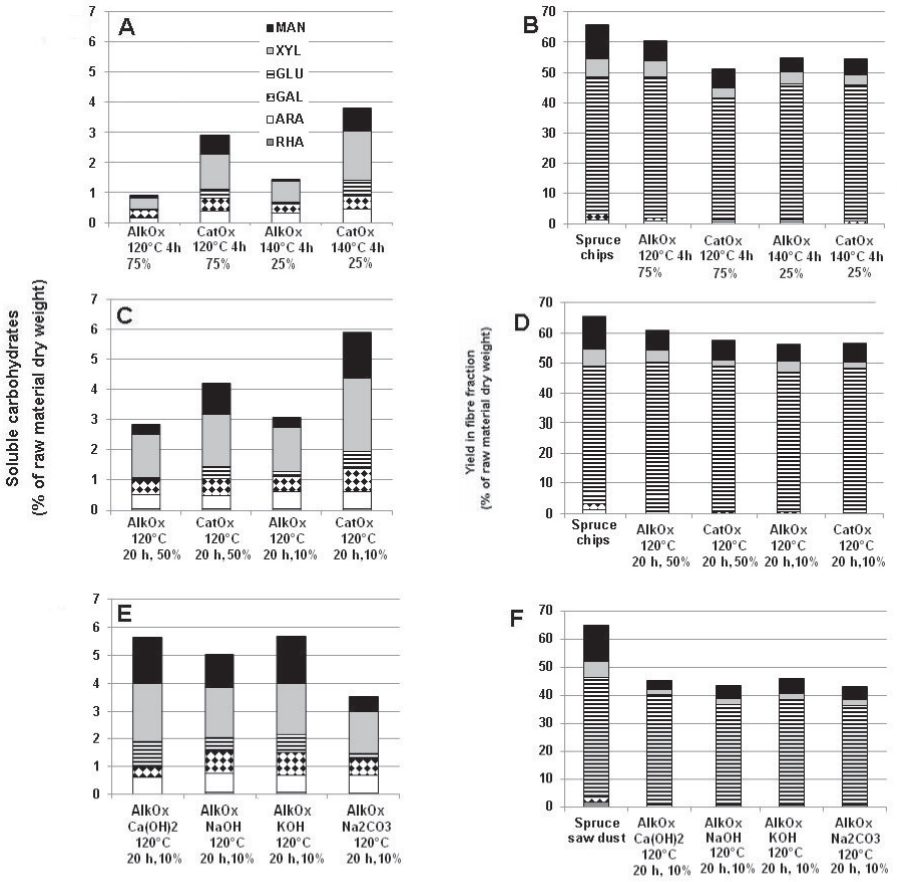

Fig. 2. Monosaccharide composition in the soluble fraction (A, C, and E) and the fibre (B, D, and F) fraction after acid hydrolysis in series I - III. The carbohydrate composition of spruce chips, wood meal, and saw dust are also presented in Figs. B, D, and F, respectively. The composition was determined by HPAEC-PAD after acid hydrolysis and presented as % (w/w) of monosaccharides in the original raw material. The details of the fractionation conditions are shown in Table 1.

Comparison of the SEC chromatograms obtained with Ultrahydrogel columns using UV and RI detectors reveals that polysaccharides are dissolved as small fragments of oligosaccharides with a Mw range of 600 to 1600. The AlkOx treatment favored the dissolution of larger fragments (Fig. 3). On the other hand, CatOx fractionation produced even larger oligosaccharide fragments with a Mw of 1600 to 1900, monitored with the RI detector. Moreover, for the 20 h experiments, the CatOx treatment dissolved more lignin with a Mw between 3900 and 4000 than the corresponding AlkOx treatments, as seen in the chromatograms monitored with the UV detector.

Comparison of the precipitation yields of lignin in series I and II indicates that the yields were larger in the AlkOx samples than in the CatOx samples for both the 4 h and 20 h fractionations. At longer fractionations these correlated well with the amount of dissolved lignin as presented in Fig. 1d. The lowest yields, 17.6 % and 13 %, were obtained in the CatOx samples in which the filling ratios were 25 % and 10 %, respectively. This indicates that the co-operation of catalyst and oxygen promoted lignin degradation. Moreover, the lower yield correlates with the higher amount of oxalic acid, which is the oxidation product of lignin (Fig. 4).

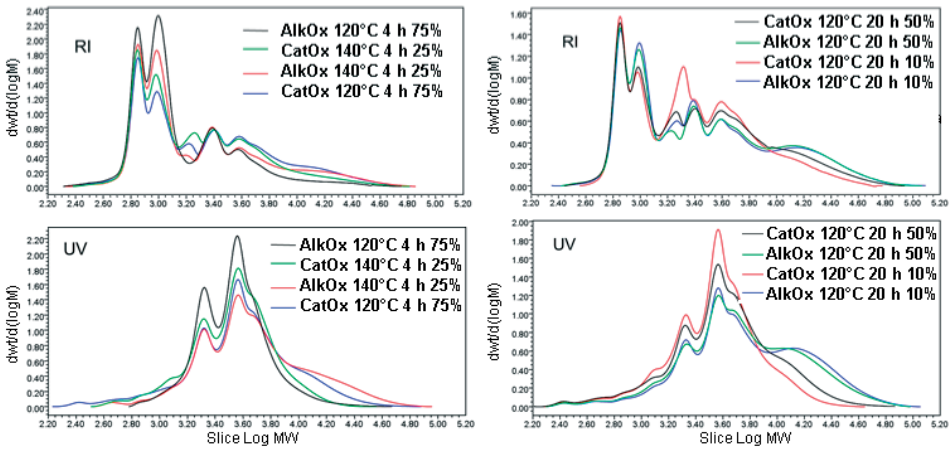

Fig. 3. SEC chromatograms of series I (left) and II (right) obtained from both the RI and UV detectors. The details of the fractionation conditions are described in Table 1.

Acids

The acidic pretreatment of lignocellulosic materials forms a variety of degradation products, which have an inhibiting effect on both the enzymatic action and the fermentation by yeast or bacteria. Among those compounds are phenols, aromatic aldehydes, soluble lignin compounds, and organic acids, such as formic and acetic acids (Sassner et al. 2006; Mosier et al. 2005; Chen et al. 2006; Hendriks and Zeeman 2009). The AlkOx and CatOx processes produce considerable amounts of the mentioned aliphatic carboxylic acids; therefore it is justified to study the nature and concentrations of the acidic compounds formed during the fractionations.

Both the AlkOx and CatOx treatments of spruce chips showed a release of lignin along with hemicelluloses. The acids were formed via the degradation of the phenolic structures of lignin and the degradation of hemicelluloses. The reactions are similar to those reactions proposed to occur in conventional oxygen delignification (Kuitunen et al. 2011; Kadla et al. 1999; Gierer 1986). The hemicelluloses are degraded through peeling reactions and chain cleavage.

Table 3. Average Molecular Weights (Mn, Mw), Polydispersities (Mw/Mn), and Precipitation Yields. *

Sample	Mw (g mol^{-1})	Mn (g mol^{-1})	PD (Mw/Mn) (g mol^{-1})	Mw (g mol^{-1})	Mn (g mol-1)	PD (Mw/Mn) (g mol^{-1})	Yield of precipitation (%)
	$NaNO_3$ /UV pullulan std			$NaNO_3$/ RI pullulan std			
AlkOx 120°C 4 h 75%	4 000	3 000	1.4	2 300	1 100	2.1	20.3
CatOx 120°C 4 h 75%	10 600	2 600	2.1	4 200	1 400	3	20.4
AlkOx 140°C 4 h 25%	7 100	3 300	2.1	3 900	1 300	3	33.9
CatOx 140°C 4 h 25%	4 100	2 600	1.6	3 300	1 300	2.5	17.6
CatOx 120°C 20 h 50%	5 700	2 600	2.2	5 200	1 600	3.3	26.7
CatOx 120°C 20 h 10%	4 500	2 500	1.8	4 100	1 600	2.6	13.0
AlkOx 120°C 20 h 50%	9 000	3 300	2.7	6 300	1 600	3.9	38.7
AlkOx 120°C 20 h 10%	9 700	3 500	2.8	6 100	1 600	3.8	40.2

* Precipitation yield was calculated using an absorptivity value of 20 L g^{-1} cm^{-1}.

The reactions of both lignin and hemicellulose with molecular oxygen, perhydroxyl/superoxide radicals (HO_2^*/O_2^{*-}), hydroperoxide anions (HOO^-), and hydroxyl radical/oxide anion radicals (HO^*/O^{*-}) cause degradation of those macromolecules and the formation of small aliphatic carboxylic acids (Gellerstedt et al. 1980; Young and Gierer 1976; Bailey and Dence 1969; Kuitunen et al. 2011).

Capillary zone electrophoresis (CZE) with indirect UV detection was used for the simultaneous determination of inorganic anions and C(1)-C(6) aliphatic carboxylic acids liberated in the lignocellulosic processed samples (Rovio et al. 2010). From the analytical point of view, the separation of the aliphatic carboxylic acids is a challenging task, since they have similar mass-to-charge ratios and similar molecular structures. The optimized method was applied for determining the carboxylic acids in the soluble fractions of the oxidation treatments.

Both volatile and non-volatile carboxylic acids were detected. The volatile acids include formic and acetic acids, while the non-volatile acids include dicarboxylic acids and hydroxy acids. The dominating acids in the experiments were formic, acetic, oxalic, and glycolic acid both in alkali-oxidized and catalytically-oxidized samples. Moreover, malonic, fumaric, succinic, malic, lactic, 3-hydroxypropionic, 2-OH-butyric, and 2.5-di-OH-pentanoic acids were found in smaller quantities (Fig. 4). In general, the acid profiles were similar in all experiments with only small variations in the concentrations of

individual acids. Most likely, the reason for this is the alkaline oxidative environment which promotes similar reactions despite the nature of the alkali.

The most crucial reaction parameters with respect to the formation of the acids were the presence of a catalyst, reactor filling, and reaction temperature. The catalytically assisted oxidation produced more acid regardless of the reaction temperature. Catalyzation had the greatest affect in the formation of formic acid and oxalic acid. Oxalic acid has been generally recognized as a degradation product of lignin (Kuitunen et al. 2011; Bailey and Dence 1969).

The total acid formation was enhanced both in the AlkOx and CatOx experiments at a temperature of 140 °C. The acid production in both treatments was balanced with a reaction time of 20 hours; however, the lower reactor filling (10% instead of 50%) had a positive effect on wood degradation and oxidation of dissolved lignin and hemicelluloses, leading to the increase in acid formation from *ca*. 15% to 20% (Fig. 4). Comparison of bases showed similar acid productions, but slightly more oxalic and minor acids were formed in samples treated with Na_2CO_3, NaOH, and KOH than with $Ca(OH)_2$, which in turn favors the generation of formic and acetic acids.

The composition of the major acids was similar to what was observed in the wet oxidation of wheat straw (Bjerre et al.1996) and the mixture of clover and ryegrass (Martín et al. 2008). In addition, Samuelson and Sjöberg (1976) studied spent liquor obtained from oxygen bicarbonate cooking of birch and found the same major acids: formic, acetic, oxalic and glycolic acids. Shuai et al. (2010) observed that dilute acid and SPORL pretreatments produced formic acid levels of 7.4 and 1.9 g L^{-1}, respectively, whereas the amount of formed acetic acid were 5.3 and 2.7 g L^{-1}, respectively. Moreover, Larsson et al. (1999) reported, that steam explosion of acid treated spruce chips produced 1.6 g L^{-1} of formic acid and 2.4 g L^{-1} of acetic acid. It should be noted that acidic pretreatments produce levulinic acid, which is a potent inhibiting compound too (Palmqvist and Hahn-Hägerdal, 2000). In our study the concentrations of formic acid and acetic acid varied within the ranges 0.8 to 2.5 g L^{-1} and 0.9 to 3.3 g L^{-1}, respectively.

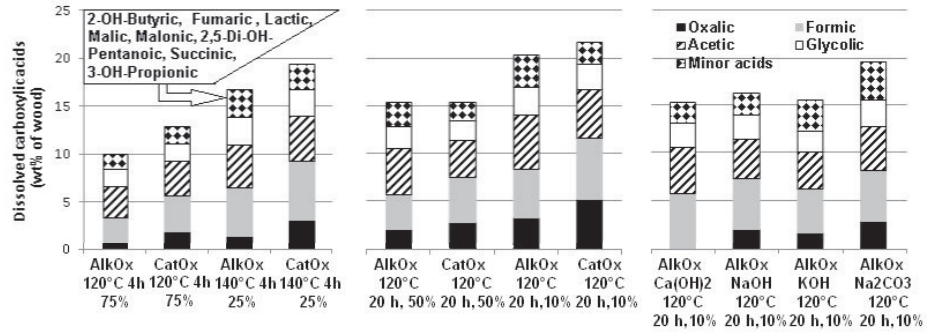

Fig. 4. Distribution of four main aliphatic acids and sum of minor acids found in the soluble fraction in series I - III after oxidation. Acid composition was determined by capillary electrophoresis as described in Chapter Methods and presented as % acid from the original raw material. The details of fractionation conditions are described in Table 1.

Kinetics of Catalyzed Oxygen Delignification

The progress of the reaction was monitored by analyzing the dissolved material as a function of time.

Analysis of the samples of the CatOx reaction mixture at predetermined intervals showed increased dissolution of hemicelluloses after one hour of oxidation (Fig. 5, center). After four hours of treatment the carbohydrates were solubilized more slowly. The content of carbohydrates in the fiber fraction originating from hemicellulose decreased by half of its original concentration during 20 hours of the reaction. The cellulose was not dissolved significantly, as the concentration of glucose decreased by only five per cent (Fig. 5, left).

Almost half of the acetic acid and formic acid was generated during the first hour, and such generation continued over the course of the oxidation. The formation of glycolic, oxalic, and 2,5-di-OH-pentanoic acids was more vigorous during the first four hours and became slower thereafter (Fig. 5, right).

The dissolved lignin content was estimated based on UV analysis at 280 nm. For aromatic lignin, an absorptivity of 20 L g^{-1} cm^{-1} was used. In addition, the absorptivities of the isolated (unpurified) lignins were determined. Table 4 shows the yields of dissolved lignin based on the UV results. The content of dissolved aromatic lignin followed a similar dissolution pattern as the hemicelluloses, while the majority of the dissolution of lignin occurred during the first four hours of the reaction, and only minor changes were seen between 4 and 20 hours. About half of the dissolved lignin was precipitated by the acidification, and this proportion was not affected by the stage of the reaction. The precipitation yields in the kinetic series were larger than those of the AlkOx and CatOx samples (Table 3).

The formation of carboxylic acids was supposed to occur *via* both the oxidation of dissolved hemicelluloses and the degradation of aromatic lignin structures. It was presumed that the oxidation of lignin affected the absorptivity of the dissolved lignin. Surprisingly, a decrease in the absorptivity was not observed, but rather a slight increase.

Part of the fiber components may have dissolved in the form of degradation products and were not detected by the methods applied; therefore, the total content of dissolved organic material was reported as total organic carbon (TOC), and the contributions of lignin and carbohydrates to TOC were calculated using carbon contents of 0.60, 0.44, and 0.40 for lignin, carbohydrates, and carboxylic acids, respectively (Laine and Tamminen 2002).

Table 4. Precipitated Lignin, Dissolved Lignin, Precipitation Yield, and Absorptivity

Time (h)	Precipitated lignin, mg g^{-1}	Dissolved lignin, mg g^{-1}	Precipitation yield, %	Absorptivity of lignin precipitate, L g^{-1} cm^{-1}
1	44,5	100.4	44	17.5
4	87.3	185.6	47	20.1
8	78.0	195.2	40	22.6
20	90.1	194.0	46	22.1

*Weight is based on dry wood. Precipitation yield was calculated using an absorptivity value of 20. Absorptivity of the precipitate was at 280 nm.

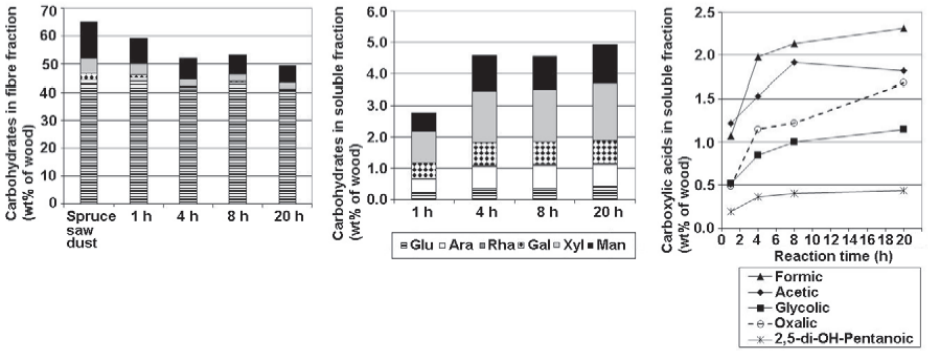

Fig. 5. The development of concentrations of different monosaccharides under 20 hours of CatOx oxidation in the fiber fraction (left) and in the soluble fraction (center). Formation of carboxylic acids under the same conditions is presented in the right figure. Details of fractionation conditions of the samples are described in Table 1, samples IV 1-4.

As seen in Fig. 6, lignin accounted for more than half the TOC, whereas the carbohydrates had only a small contribution. The contribution of carboxylic acids was approximately three times than that of the carbohydrates. About 20 to 25 percent of the TOC was composed of unidentified material.

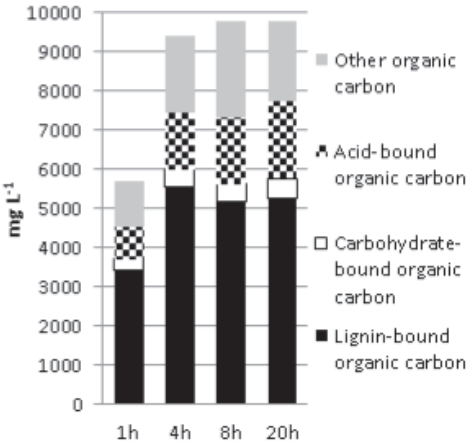

Fig. 6. Total dissolved organic carbon and its distribution between lignin, carbohydrates, and other organic material. Details of fractionation conditions of the samples are described in Table 1, samples IV 1-4.

CONCLUSIONS

Alkaline oxidation can be catalyzed by a copper-phenanthroline complex to enhance the fractionation of lignocellulosic material and decrease the reaction time. This catalyzed oxidation (CatOx) process was studied in a laboratory scale reactor in order to understand the chemistry taking place, in particular the role of the catalyst, using Spruce wood as the raw material.

1. During the catalytic pretreatment the wood chips lost their macroscopic structure, and more than 28% of the dry wood material was solubilized during the 20 hours catalytic pretreatment. Both catalytic and alkaline oxidations resulted in a cellulose-rich fiber material. The solid material contained over 83% of the original total carbohydrates after four hours of catalytic oxidation at 140 °C, which was a similar value to that of a non-catalyzed counterpart. An extended pretreatment time did not alter the total yields of the fiber fraction, being 87 to 88% in CatOx and 85 to 93% in AlkOx samples. The applicability of the fiber fraction for bioethanol production was shown in earlier studies (Hakola et al. 2010).
2. The effectiveness of the catalyst in the dissolution of lignocellulosic material was also observed by analyzing the composition of the dissolution fraction. The closer examination of dissolved compounds revealed that the catalytically assisted oxidation was more efficient than its alkaline counterpart in the dissolution of carbohydrates, especially of those originating from glucomannan and arabino-xylan, which can be verified by analyzing the proportions of mannose and xylose in the dissolved fraction. In addition, the CatOx process was more efficient with a low reactor filling ratio.
3. The catalyst increased both the degradation of dissolved monosaccharides and the degradation of lignin to small aliphatic carboxylic acids. This observation was more pronounced with reactor filling ratios below 25%. The acid profile was quite similar in the presence and absence of the catalyst, although some variations in the concentration of individual acids were observed. Comparison of the four different bases showed that NaOH, KOH, and Na_2CO_3 were comparable in dissolution efficiencies.

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PAPER VI

Fractionation of organic and inorganic compounds from black liquor by combining membrane separation and crystallization

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Research Article

Fractionation of Organic and Inorganic Compounds from Black Liquor by Combining Membrane Separation and Crystallization

Separation of organic and inorganic compounds from black liquor was investigated. Black liquor from the pulp and paper industry contains hundreds of different compounds and several high-value organic chemicals are formed during alkaline pulping. These organic compounds can be used in the fine chemicals, pharmaceutical and food industries. The main aim of this study was to recover and purify high value-added organic compounds (organic acids) from lignin and inorganic compounds by combining membrane filtration, acid precipitation and cooling crystallization. The effect of membrane filtration on the efficiency of precipitation and crystallization was also studied. A number of separation methods were studied under a variety of operating conditions. The results showed that a combination of membrane separation and crystallization is an efficient method for recovering and purifying valuable organic compounds in black liquor. The amount of major impurities in black liquor could be reduced significantly.

Keywords: Black liquor, Crystallization, Membrane processes, Organic acids, Separation

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1 Introduction

Carboxylic acids have become commercially important chemicals in the production of polymers, pharmaceuticals and food additives and they are among the most attractive products to be produced from biomass. In pulp digesting, for example, more than 20 different carboxylic acids are formed by decomposition of carbohydrates. According to Morrison and Boyd [1], most carboxylic acids are in the form of a transparent crystal solid at room temperature. Smaller carboxylic acids (1–5 carbon atoms) are miscible with water, alcohols, acetone and some lipids, whereas compounds containing more than six carbons are slightly soluble or insoluble in water. The latter ones tend to be rather soluble in nonpolar solvents, such as ethers and alcohols. The carboxylic acids present in black liquor are usually hydroxyl acids, with a carbon chain of between 2–6 carbon atoms. Tab. 1, from Sjöström and Alén [2], shows typical compositions of pine and birch black liquors.

Separation of carboxylic acids from black liquor is challenging due to low concentrations and the presence of other organic and inorganic compounds. In addition to water, black liquor contains hundreds of different compounds, such as organic residue from pulping and inorganic cooking chemicals. The primary organic compounds are lignin, carboxylic acids, polysaccharides and extractives. Tab. 2 gives a typical analysis of kraft black liquor, adapted from Sjöström and Alén [2].

Separation of hydroxy acids from black liquor has been studied since the 1980s. The processes that have been developed are based on precipitation of high molar mass lignin and purification of the filtrate using chromatographic methods. Precipitation of lignin is usually performed with carbon dioxide and/or sulfuric acid because lignin has a lower solubility in acidic solutions than in solutions with a higher pH. Precipitation and filterability of lignin from black liquor has been studied by Öhman and Theliander [3], Johansson et al. [4] and Moosavifar et al. [5]. It has been shown that temperature, pH value, and ionic strength are the most important parameters influencing the solubility of lignin in black liquor. Yields of precipitated lignin increase with decreasing pH. The results show that lignin starts to precipitate at about pH 11.5. Toledano et al. [6–8], Garcia et al. [9], and González Alriols et al. [10] have studied selective precipitation, membrane separation and characterization of lignins.

The residual liquor containing hydroxy acids can be purified with distillation. However, this approach has some disadvantages. For instance, lactic acid, glycolic acid and 2-hydroxybutanoic acid cannot be separated from each other by dis-

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Table 1. Main carboxylic acids present in black liquor with typical approximate concentrations, adapted from [2].

Monocarboxylic acids		
Acid	Pine concentration [g L^{-1}]	Birch concentration [g L^{-1}]
Glycolic	3	2
Lactic	4	4
3-Hydroxypropanoic	0.02	0.19
Glyceric	0.13	0.11
2-C-Methylglyceric	0.10	0.12
2-Hydroxybutanoic	1.0	7
4-Hydroxybutanoic	0.19	0.10
2-Deoxytetronic	0.09	0.10
3-Deoxytetronic	0.3	0.6
2-Hydroxypentenoic	0.3	0.15
3,4-Dideoxypentonic	2.2	1.2
2,5-Dihydroxypentanoic	1.5	0.9
Xyloisosaccharinic	0.5	4
Anhydroisosaccharinic	0.3	0.18
3,6-Dideoxyhexonic	0.16	0.6
3-Deoxyhexonic	0.3	0.3
Glucoisosaccharinic	9	4
Dicarboxylic acids		
Oxalic	0.13	0.17
Succinic	0.22	0.22
Methylsuccinic	0.18	0.04
Malic	0.16	0.3
2-Hydroxyglutaric	0.4	0.5
3-Deoxypentaric	0.05	0.07
2-Hydroxyadipic	0.4	0.24
2,5-Dihydroxyadipic	0.4	0.22
Glucoisosaccharinaric	0.5	0.7

tillation because their boiling points are very close to each other.

A new approach to the separation and purification of hydroxy acids from black liquor might be a combination of membrane separation and crystallization. The use of membranes in the separation of lignin from black liquor might be advantageous because colloids are formed in precipitation with mineral acids, causing difficulties in further processing of the black liquor, and the obtained lignin fraction has low purity [8]. With membrane technology lignin fractions with defined molar masses can be produced and the fractions have a higher purity compared to the fractions obtained by precipitation.

Table 2. Composition of black liquor dry solids from kraft pulping of pine, adapted from [2].

Organics, [wt-%]	67
Lignin, [wt-%]	31
Aliphatic carboxylic acids, [wt-%] – Hydroxyl monoacids – Hydroxyl diacids	29
Other organics, [wt-%] – Polysaccharides – Extractives – Miscellaneous	7
Inorganics, [wt-%]	33
NaOH	
Na_2S	
Na_2CO_3	
Na_2SO_4	
Na_2SO_3	
$Na_2S_2O_3$, NaCl	
Others	

Crystallization of inorganic compounds from acidified black liquor has not been reported. The present work introduces a crystallization method by which the content of inorganic salts can be reduced. A high inorganic content in the mother liquor usually disturbs separation processing of fine chemicals. The aim is to recover and purify hydroxy acids from black liquor by combining membrane filtration, acid precipitation, and crystallization processes.

2 Experimental Methods

2.1 Black Liquor

The black liquor used in the experiments was from a kraft pulp mill which uses both softwood and hardwood as raw material in the pulping process. The dry solids content was about 250 g L^{-1} and the pH around 13.5. The lignin content was approximately 82 g L^{-1}. The experiments were performed using undiluted black liquor and the amount of identified organic acids was 37 g L^{-1}.

2.2 Membrane Separation

The membrane filtration experiments were performed with a DSS LabStak M20 filter manufactured by Alfa Laval. This equipment is a flat sheet type filter in which several membrane sheets can be tested in series at the same time. The filtration area of one membrane sheet is 0.036 m^2. The permeates of the membranes are collected separately.

The membranes used in the experiments were the ultrafiltration (UF) NP010 membrane and the nanofiltration (NF)

NP030 membrane from Microdyn-Nadir GmbH. Their cut-off values were 1000 and 400 Da, respectively. The membranes were made from polyethersulfone. According to Microdyn-Nadir [11], the maximum operational temperature of the membranes is 95 °C and they can be used at a pH range of 0–14.

The membranes were pretreated with alkaline cleaning solutions (20 min at 40 °C, 0.1 wt-% P3-ultrasil 110 from Ecolab) and then pressurized with water at 30 bar for 1 h. To observe possible changes in membrane performance, pure water flux was measured after pre-treatment, after filtration of black liquor, and after cleaning. The cleaning was carried out with an alkaline solution (1.0 wt-% P3-ultrasil 110) at 70 °C.

Lignin removal efficiency was tested for both the NP010 and NP030 membranes. The filtration with NP010 was performed at 15 bar with a cross-flow velocity of 1.4 m s^{-1} and it was continued until the feed volume reduction was 67 %. A pressure of 12 bar was used in the filtration with the NP030 membrane and the cross-flow velocity was 1.5 m s^{-1}. This filtration experiment was continued until the feed volume reduction was 60 %.

2.3 Crystallization

The crystallization experiments were carried out in a 2-liter jacketed stirred tank with a three-bladed marine propeller and profiled bottom. The solution temperature was adjusted with a thermostat.

The precipitation experiments performed with raw black liquor and NF permeate at 25 °C. To remove lignin from the solutions, the pH was adjusted by adding sulfuric acid. The final pH value of the solutions was 2. Cooling crystallization and antisolvent precipitation were tested for the solutions obtained after the addition of sulfuric acid.

2.4 Analysis

The ratio of inorganic and organic compounds in the crystals obtained and the residual mother liquor was analyzed using a high temperature oven (supplier Nabertherm). The analysis of the lignin removal efficiency of the membrane processes was based on UV absorption at 280 nm (measured with a Varian Cary 1C spectrophotometer). The absorption constant used was 24.7 L $g^{-1}cm^{-1}$, from Sundin [12], which was determined for pure softwood. The black liquor used in the experiments was mainly from softwood and partly from hardwood. Thus, the lignin concentrations obtained with the absorption constant value shown above are not exact values but good estimates.

Analyses of individual carboxylic acids were carried out by capillary electrophoresis. Separations of the carboxylic acid standards and ultrafiltration samples were performed with a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a photodiode array UV detector. Details of the background electrolyte (BGE) and performance of the separation are presented in [13]. Uncoated fused silica capillaries of 50 µm I.D. and 50/60 cm length (effective length/total length) were employed. The samples were injected at a pressure of 0.5 psi (34.5 mbar) and the injection time was set to 5 s. The capillary and samples were thermostatted to +15 °C. The detection wavelength was set to 281 nm using an indirect detection mode. Before the measurements, the new capillaries (from Teknolab Trollåsen, Norway) were conditioned by rinsing sequentially with 0.1 M sodium hydroxide, 0.1 M HCl and ultrapure water, each solution for 20 min, and then with electrolyte solution for 5 min. Between analyses, the capillaries were rinsed with 0.1 M HCl for 3 minutes, 0.1 M NaOH solution for 1 min, and the electrolyte solution for 5 min, all at a pressure of 20 psi (1380 mbar). Prior to the CE analyses, the samples were diluted 1:50 and 1:100 (v/v) with 20 mM NaOH in order to enhance the ionization of the analytes and to ensure opening of the lactone structures present in glucoisosaccharinic acid.

3 Results and Discussion

The purpose of this study was to combine membrane separation and crystallization in the separation of carboxylic acids from black liquor. Raw black liquor and the permeate of nanofiltration of a black liquor were used as the raw materials for crystallization. The obtained NF permeate fraction contains mainly carboxylic acids, smaller lignin compounds, and inorganic salts. An essential part of the experimental work was to find a method to remove lignin and inorganic compounds from solutions containing carboxylic acids.

3.1 Membrane Separation

The results revealed that membrane filtration removed the main part of the lignin. It was found that nanofiltration with the NP030 membrane reduced the lignin content from 82 g L^{-1} to 18 g L^{-1}. The permeate flux through the NP030 membrane was, however, significantly lower compared to the permeate flux through the NP010 membrane, as shown in Fig. 1. UF with the NP010 membrane reduced the lignin content from 82 g L^{-1} to 20 g L^{-1}. The hydroxy acids permeated the membranes.

3.2 Crystallization

3.2.1 Acid Precipitation of Lignin

Preliminary experiments indicated that lignin makes the separation of carboxylic acids by crystallization very difficult. It was therefore assumed that removal of lignin from the solution could enhance the efficiency of downstream processing. According to Öhman and Theliander [3], the pH value of the black liquor is the most important parameter controlling the concentration of lignin in the black liquor. The effect of pH on the removal of lignin by precipitation was studied and pH 2 was observed to be the optimal point to minimize the lignin concentration in the solution. The addition of more sulfuric acid only slightly increased the crystal mass. The crystals were filtered with a Büchner funnel. After the acid precipitation, the

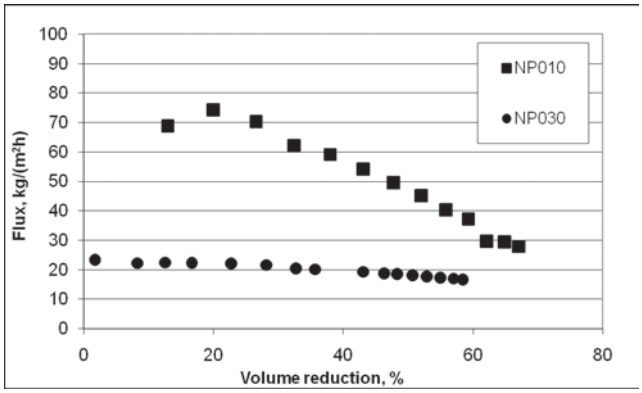

Figure 1. Permeate flux in filtration of raw black liquor with membranes NP010 (at 15 bar) and NP030 (at 12 bar). Filtrations were performed at 70 °C.

lignin concentration in the residual mother liquor was below 6.3 g L^{-1}.

3.2.2 Cooling Crystallization

Cooling crystallization was tested as a method of separating inorganic matter from the solution. The experiments were carried out with raw black liquor and NF permeate from which the lignin had been precipitated at the pH presented in Sect. 2.3. The results of cooling crystallization presented in Tab. 3 were obtained by cooling to a final temperature of –4 °C. The organic acid concentrations of the solutions before and after crystallization were analyzed. The initial concentration of organic acids of the raw black liquor solution and the NF permeate were about 44 g L^{-1}. After cooling crystallization, the organic acid concentration remained in a range of ca. 34–40 g L^{-1}. The concentration of inorganic compounds decreased remarkably after cooling crystallization, as can be seen from the sulfate ion concentrations in Tab. 3.

The studied raw black liquor and NF permeate solutions are compared in a following section. The initial lignin content of the raw black liquor was 82 g L^{-1}. The NF treatment was carried out with a 400 Da membrane, resulting in a lignin concentration of the permeate of 18 g L^{-1}. The initial content of organic acids in the raw black liquor and the permeate was 37 g L^{-1} and 44 g L^{-1}, respectively. The concentration of organic acids was slightly higher in the NF permeate than in the original black liquor, i.e., acids retention was negative. Both the lignin and organic acid molecules were negatively charged in the filtration at pH 13.5. The Donnan exclusion mechanism might, therefore, be the cause of the negative retention of organic acids since negatively charged higher molar mass lignin was well retained. The used volume reduction of 60 % was rather low, and about 30 % of the original organic acids were still in the concentrate stream. A higher yield of organic acids into NF permeate can be achieved by increasing the volume reduction in the filtration. Theoretically, at 80 % volume reduction, 95 % of organic acids will be in the permeate fraction. At 60 % volume reduction about 85 % of the original lignin was recovered into the concentrate stream. The lignin concentration in the concentrate was around 230 g L^{-1}. Less than 15 % of sulfate ions were retained by NF. Therefore, it is evident that most of the inorganic cooking chemicals (sodium, and sulfur) passed through the NF membrane.

As shown in Tab. 3, the composition of raw black liquor differs clearly from the NF permeate composition which leads also to different kind of separations by crystallization. Concentrations of lignin and organic acids after cooling crystallization slightly decreased when treating the raw black liquor, whereas

Table 3. Crystallization of raw black liquor and nanofiltered black liquor. Concentrations of lignin, sulfate, formiate and acetate ions, and organic acids of the feed solution and the solutions after each separation step (acid precipitation of lignin, cooling crystallization, and antisolvent treatment).

Separation step / Conc. [g L^{-1}]	Lignin	Sulfate	Formic	Acetic	Glycolic	Lactic	2-OH-butyric	2,5-Di-OH-pentanoic	Xisa[a)]	Gisa[b)]	Acids together
Black liquor (raw)	82	7.5	10	6.7	2.8	5.1	1.2	1.1	4.2	5.8	37
Acid precip. filtrate	4.4	6.7	13	8.2	2.9	5.3	0.9	1.0	5.5	6.8	44
Cooling cryst. filtrate	4.0	2.1	9.2	5.9	2.7	4.7	1.1	0.7	4.0	5.5	34
Solvent precip. filtrate[c)]	2.4	1.6	3.7	2.7	1.2	2.0	0.6	0.5	1.8	2.3	15
Black liquor (NF perm.)	18	6.4	13	7.8	3.3	5.7	1.5	1.1	4.7	6.7	44
Acid precip. filtrate	6.1	3.1	11	7.0	2.4	4.4	1.0	0.8	4.5	4.7	36
Cooling cryst. filtrate	7.3	1.6	11	7.6	3.3	5.6	1.3	1.0	4.5	5.9	40
Solvent precip. filtrate[c)]	3.8	0.6	4.5	3.7	1.5	2.6	0.6	0.6	2.2	2.9	19

[a)]xyloisosaccarinic, [b)]glucoisosaccarinic, [c)]added 50 wt-% solvent

in the NF permeates these concentrations increased. The increase in concentrations may be explained by the greater hydrate formation by the crystallization of inorganic compounds from NF permeates.

Concentration of other organic compounds, e.g., polysaccharides, in the different stages of this separation process was not analyzed. However, polysaccharides are degraded to smaller compounds (e.g., organic acids) during the cooking process, and thus, their amount in black liquor is small. Therefore, their effect on the separation of lignin and organic acids is most probably not very significant.

3.2.3 Addition of Antisolvent

The results of the cooling crystallization experiments showed that a small amount of inorganic salts is still present in the solutions. It is known that the solubility of inorganic compounds in a mixture of an organic solvent and water is very low and decreases with increasing organic mass fraction in the solvent. For this reason, further removal of the inorganic compounds was attempted with the addition of an organic solvent.

The solutions used in this step were the solutions initially obtained from the raw black liquor and the NF permeate, which had been treated by acid treatment and cooling crystallization. The final mass fraction of organic solvent in the mixture was 50 %. The organic solvent was not capable of removing any further lignin from the solution. In addition, the removal of sulfates (inorganic compounds) was not remarkable.

3.2.4 Summary

Fig. 2 gives a proposal for a separation process using a combination of membrane filtration and crystallization. The proposed process will purify hydroxy acids as far as their content in dry solids is above 50 %. Most of the lignin is recovered by the UF/NF stage as a concentrated solution (~230 g L^{-1}). Further purification and concentration of hydroxy acids could be done by tight nanofiltration or chromatography.

4 Conclusions

Separation of carboxylic acids from black liquor is challenging due to the presence of numerous organic and inorganic compounds. Lignin and inorganic salts inhibit efficient separation of other high-valued organic compounds from black liquor. Combining membrane separation methods with crystallization was investigated with the aim of developing an efficient separation process for the fractionation of lignin and inorganic salts, and for retention of carboxylic acids with maximum recovery in the residual liquor. The solutions used in the crystallization experiments were raw black liquor and permeate obtained from membrane filtration.

Several crystallization methods were tested under alkaline conditions for black liquor without success. Acidification of black liquor precipitated lignin and the residual liquor could be further purified by cooling crystallization, which removes 50–70 % of the salts. Further treatment by adding an organic solvent to the delignified solutions led to only a slight increase in removed inorganic compounds.

Combining membrane filtration and crystallization, the initial content of lignin and inorganic compounds in black liquor could be reduced by 90–95 % and 70–75 %, respectively. A single nanofiltration or ultrafiltration step removed about 85 % of lignin when permeate recovery was 60 %. The product of this process is a mixture of several carboxylic acids. In the studies performed here, glucoisosaccharinic acid was the major compound of the organic acids in the final solution. Based on the obtained results, the isolation processes of the desired organic compounds can be enhanced based on the combination of membrane filtration and crystallization.

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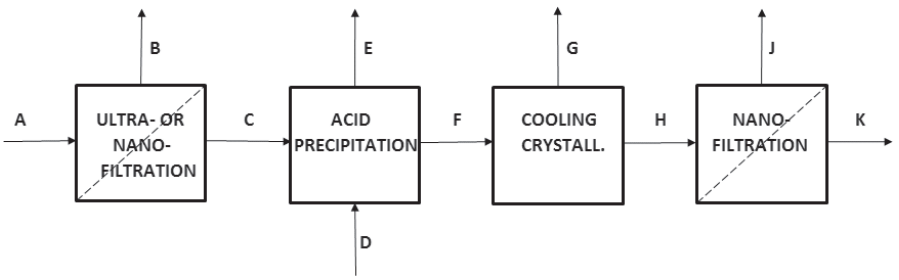

Figure 2. Process flow sheet for separation of carboxylic acids from black liquor. The flows are as follows: A: raw black liquor, B: retentate, C: permeate, D: concentrated sulfuric acid, E: lignin precipitate, F: filtrate solution, G: inorganic crystals, H: filtrate solution, J: product (retentate solution, concentrated organic acid solution), K: permeate (solvents).

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Series title and number
VTT Science 21

Title	**Application of capillary electrophoretic methods for determining carbohydrates and aliphatic carboxylic acids formed during wood processing**
Author(s)	Stella Rovio
Abstract	Knowledge of the behaviour of cellulose, hemicelluloses, and lignin during wood and pulp processing is essential for understanding and controlling the processes. Determination of monosaccharide composition gives information about the structural polysaccharide composition of wood material and helps when determining the quality of fibrous products. In addition, monitoring of the acidic degradation products gives information of the extent of degradation of lignin and polysaccharides. This work describes two capillary electrophoretic methods developed for the analysis of monosaccharides and for the determination of aliphatic carboxylic acids from alkaline oxidation solutions of lignin and wood. Capillary electrophoresis (CE), in its many variants is an alternative separation technique to chromatographic methods. In capillary zone electrophoresis (CZE) the fused silica capillary is filled with an electrolyte solution. An applied voltage generates a field across the capillary. The movement of the ions under electric field is based on the charge and hydrodynamic radius of ions. Carbohydrates contain hydroxyl groups that are ionised only in strongly alkaline conditions. After ionisation, the structures are suitable for electrophoretic analysis and identification through either indirect UV detection or electrochemical detection. The current work presents a new capillary zone electrophoretic method, relying on in-capillary reaction and direct UV detection at the wavelength of 270 nm. The method has been used for the simultaneous separation of neutral carbohydrates, including mono- and disaccharides and sugar alcohols. The in-capillary reaction produces negatively charged and UV-absorbing compounds. The optimised method was applied to real samples. The methodology is fast since no other sample preparation, except dilution, is required. A new method for aliphatic carboxylic acids in highly alkaline process liquids was developed. The goal was to develop a method for the simultaneous analysis of the dicarboxylic acids, hydroxy acids and volatile acids that are oxidation and degradation products of lignin and wood polysaccharides. The CZE method was applied to three process cases. First, the fate of lignin under alkaline oxidation conditions was monitored by determining the level of carboxylic acids from process solutions. In the second application, the degradation of spruce wood using alkaline and catalysed alkaline oxidation were compared by determining carboxylic acids from the process solutions. In addition, the effectiveness of membrane filtration and preparative liquid chromatography in the enrichment of hydroxy acids from black liquor was evaluated, by analysing the effluents with capillary electrophoresis.
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Julkaisun sarja ja numero
VTT Science 21

Nimeke	**Kapillaarielektroforeettisten erotusmenetelmien soveltaminen puun prosessoinnissa syntyvien sokerien ja alifaattisten karboksyylihappojen määrityksessä**
Tekijä(t)	Stella Rovio
Tiivistelmä	Käsitys selluloosan, hemiselluloosan ja ligniinin käyttäytymisestä puun käsittelyssä on oleellista, jotta prosesseja kyetään kontrolloimaan. Monosakkaridikoostumuksen määrittäminen antaa tietoa puun polysakkaridikoostumuksesta ja auttaa arvioimaan kuitutuotteiden laatua. Lisäksi happamien hajoamistuotteiden määrittäminen antaa tietoa ligniinin ja polysakkaridien hajoamisesta. Tässä työssä kuvataan kahden kapillaarielektroforeettisen menetelmän kehitys monosakkaridien ja alifaattisten karboksyylihappojen määrittämiseksi ligniinin ja puun alkalihapetuksella käsitellyistä liuoksista. Kapillaarielektroforeesi (CE) monine variaatioineen on vaihtoehtoinen erotustekniikka kromatografisille erotustekniikoille. Kapillaarivyöhyke-elektroforeesissa (CZE) silikakapillaari on täytetty elektrolyyttiliuoksella. Erotusjännite muodostaa sähkökentän kapillaarinpäiden välille, jolloin ionien liikkuvuus sähkökentässä perustuu niiden varaukseen ja hydrodynaamiseen säteeseen. Sokeriyhdisteiden hydroksyyliryhmät ionisoituvat vain hyvin emäksisissä olosuhteissa. Ionisoituminen helpottaa hiilihydraattien elektroforeettista analyysia, ja analyyttien monitorointi voidaan tehdä joko epäsuoraa UV-detektiota tai sähkökemiallista monitorointia käyttäen. Tutkimuksessa esitellään uusi kapillaarielektroforeettinen erotusmenetelmä sokerianalytiikkaan. Menetelmässä käytetään vahvasti emäksistä elektrolyyttiliuosta ja sokeriyhdisteet monitoroidaan suoraa UV-detektointia käyttäen 270 nm aallonpituudella. Käytetyt erotusolosuhteet ovat sellaiset, että mono- ja disakkaridit sekä sokerialkoholit varautuvat negatiivisesti ja niistä muodostuu UV-absorboivia yhdisteitä. Optimoitua menetelmää testattiin todellisilla näytteillä, joista mitattiin valikoitujen hiilihydraattien pitoisuudet. Tutkielmassa esitetty erotusmenetelmä on nopea, koska ainoa esikäsittelyvaihe ennen analyysia on laimentaminen. Alifaattisten karboksyylihappojen määrittämiseksi emäksisistä prosessiliuoksista kehitettiin uusi kapillaarielektroforeettinen erotusmenetelmä. Tavoitteena oli luoda menetelmä, jolla kyetään erottamaan yhdellä ajolla dikarboksyylihapot, hydroksihapot sekä haihtuvat hapot, joita syntyy ligniinin ja puun polysakkaridien hajoamis- ja hapettumisreaktioissa. Kehitettyä CZE-menetelmää sovellettiin kolmessa prosessissa. Ensimmäinen applikaatio käsitti karboksyylihappojen määrittämisen prosessiliuoksesta, kun ligniiniä hapetettiin alkalisessa liuoksessa. Toisessa sovelluksessa verrattiin katalysoitua alkalista hapetusta ei-katalysoituun alkaliseen hapetukseen määrittämällä alifaattisia karboksyylihappoja prosessiliuoksista. Karboksyylihappomäärityksiä käytettiin myös fraktioanalyyseissä tutkittaessa kalvosuodatuksen ja preparatiivisen nestekromatografian käyttökelpoisuutta karboksyylihappojen rikastamisessa mustalipeänäytteistä.
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Application of capillary electrophoretic methods for determining carbohydrates and aliphatic carboxylic acids formed during wood processing

This work describes two capillary electrophoretic methods developed for the analysis of monosaccharides and for the determination of aliphatic carboxylic acids from alkaline oxidation solutions of lignin and wood.

Capillary electrophoresis (CE), in its many variants is an alternative separation technique to chromatographic methods. Carbohydrates contain hydroxyl groups that are ionised only in strongly alkaline conditions. After ionisation, the structures are suitable for electrophoretic analysis and identification through either indirect UV detection or electrochemical detection.

The current work presents a new capillary zone electrophoretic method, relying on in-capillary reaction and direct UV detection at the wavelength of 270 nm. The method has been used for the simultaneous separation of neutral carbohydrates, including mono- and disaccharides and sugar alcohols.

A new method for aliphatic carboxylic acids in highly alkaline process liquids was developed. The goal was to develop a method for the simultaneous analysis of the dicarboxylic acids, hydroxy acids and volatile acids that are oxidation and degradation products of lignin and wood polysaccharides. The CZE method was applied to three process cases.

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