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Efficient capillary zone electrophoretic separation of wood-derived neutral and acidic mono- and oligosaccharides

Annika Rydlund, Olof Dahlman*

Swedish Pulp and Paper Research Institute, P.O. Box 5604, S-11486 Stockholm, Sweden

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Abstract

Neutral and acidic monosaccharides, commonly present as structural units in wood-derived hemicelluloses, were derivatized by reductive amination using 6-aminoquinoline (6-AQ) and subsequently separated as their borate complexes by capillary zone electrophoresis. By using a quite concentrated (420 mmol l^{-1}) alkaline borate buffer, a fused-silica capillary column with a small inner diameter (30 μm nominal I.D.) and a constant power of 1200 mW (corresponding to an applied voltage of approximately 21 kV), optimal separation was achieved. Under these conditions, the monosaccharides investigated were separated with a resolution, R_s, of 1.0–1.2 or greater. On-column UV detection at 245 nm was found to provide highly sensitive detection of the 6-AQ-derivatized monosaccharides. The minimum detectable concentrations were on the order of 10^{-6} mol l^{-1} (corresponding to an estimated limit of detection of a few fmol). The linear calibration range of the method, including the 6-AQ derivatization step, was found to be about two orders of magnitude. Several neutral β(1-4)-D-xylooligomers and acidic oligosaccharides containing 4-O-methyl-D-glucuronic acid units, which are common structural elements in hemicelluloses such as birch and spruce xylan, were also efficiently separated as 6-AQ derivatives, using the same buffer system. Finally, the usefulness of this analytical method has been demonstrated using a spruce wood xylan sample subjected to chemical and enzymatic hydrolysis.

Keywords: Wood; Derivatization; Electrophoresis; Monosaccharides; Oligosaccharides; Saccharides; Hemicelluloses; Xylan

1. Introduction

Cellulose and different hemicelluloses are the main carbohydrate constituents of wood pulp fibers [1]. Cellulose is a homopolymer of β-(1-4)glycosidically linked D-glucose units, whereas the hemicelluloses are heteroglycans containing several different types of neutral (pentoses and hexoses) and acidic (uronic acids) monosaccharides as structural elements [1]. Although considerably less abundant than cellulose, the hemicelluloses contribute significantly to the chemical and physical properties of the fibers in chemical pulps [2]. The chemical composition and structures of the hemicelluloses present may thus, at least in part, account for some of the observed differences in the properties of chemical pulps from different origins (e.g., different wood species and/or manufacturing processes).

Although the basic chemical structure of wood and pulp hemicelluloses has been known for a number of years, much interest has recently been directed towards the chemistry of pulp hemicelluloses. The

*Corresponding author.

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introduction of new or modified technologies for producing chemical pulps has stimulated studies on hemicelluloses obtained from kraft cooking [3,4], chemical bleaching [4–6] and enzymatic bleaching [7–9]. As a consequence, there is a need for new, more rapid and reliable analytical methods for the chemical characterization of hemicelluloses originating from pulp fibers and related samples.

In addition to gas–liquid chromatography [10], analytical methods based on reversed-phase high-performance liquid chromatography (HPLC) [11] or anion-exchange HPLC [12,13] have been used for the separation and determination of carbohydrates originating from wood or pulp fibers. HPLC techniques have also been employed in the analysis of partially degraded oligosaccharides originating from hemicelluloses [11,14]. Recently, capillary zone electrophoresis (CZE) has been reported to be a very powerful analytical separation technique [15–17].

The application of CZE to the analysis of carbohydrates has been reviewed by Novotny and Sudor [18] and by El Rassi and Nashabeh [19]. In comparison to HPLC, capillary electrophoresis generally offers superior separation and considerably faster analytical run times. However, in contrast to HPLC, there are only a few detection methods for CZE which are commercially available. In commercial CZE instruments, UV absorption is by far the most widely used method of detection. Although UV detection of the borate complexes of underivatized carbohydrates at 195 nm has been reported [20], more sensitive UV detection of carbohydrates can usually be achieved by precolumn derivatization with a suitable chromophore [18,21].

Huber et al. [22] recently described the precolumn derivatization of several monosaccharides originating from plant hemicelluloses using *p*-aminobenzoic acid. These authors [22] described the separation and simultaneous determination of a number of monosaccharides occurring as structural elements in the plant hemicelluloses investigated by CZE. However, the CZE system that they used failed to separate the derivatives of mannose and glucose, two frequently occurring structural elements in wood-derived hemicelluloses. Furthermore, the peak containing rhamnose, which is also quite a common component of wood-derived hemicelluloses, was found to overlap with the peak originating from the derivatization reagent (*p*-aminobenzoic acid). Consequently, rhamnose, mannose and glucose could not be determined simultaneously by CZE under the conditions that they used [22].

The aim of this study has been to develop a rapid and simple analytical procedure, based on CZE, for the separation of neutral and acidic mono- and oligosaccharides obtained upon hydrolysis of softwood and hardwood hemicelluloses. One requirement was that the CZE method developed should enable us to separate (and simultaneously quantify) the monosaccharides D-xylose, L-arabinose, L-rhamnose, D-mannose, D-glucose, D-galactose, 4-O-methyl-D-glucuronic acid (4-O-Me-D-GlcA), D-glucuronic acid and D-galacturonic acid. In our search for the appropriate conditions for such a CZE-based separation, we investigated the possible usefulness of precolumn derivatization of the carbohydrates by reductive amination with the UV chromophoric reagent, 6-aminoquinoline (6-AQ).

Previously, Nashabeh and El Rassi [23] have applied reductive amination with 2-aminopyridine and 6-AQ to the analysis of some neutral oligosaccharides by CZE in an acidic phosphate buffer system. These investigators found that the oligosaccharides labeled with 6-AQ yielded an eight-fold stronger UV signal than did those labeled with 2-aminopyridine. For this reason, we selected 6-AQ as the UV chromophoric reagent. Furthermore, since we were aiming at separations of neutral monosaccharides, we focused on alkaline borate buffer systems in our attempts to establish the optimal conditions for separation by CZE. The ability of borate to form negatively charged complexes with carbohydrates in alkaline solutions can be utilized to achieve highly efficient electrophoretic separations of both neutral and acidic monosaccharides [20,24].

The application of our optimized CZE conditions for the analytical separation of some xylose-containing oligosaccharides originating from wood hemicelluloses is briefly described. Finally, quantitative analysis of the neutral and acidic monosaccharides in two different hydrolyzates of a spruce wood arabino-(4-O-methyl-α-D-gluco-pyranosyl-uronic acid)-xylan sample is presented.

2. Experimental

2.1. Instrumentation

CZE analyses were performed using a Dionex capillary electrophoresis system (Sunnyvale, CA, USA), equipped with a variable-wavelength UV–Vis detector (190–800 nm). UV detection was performed at 245 nm with the detector placed at the cathode, 5 cm from the end of the capillary column. Two different uncoated fused-silica capillary columns were used. The first capillary column had a total length of 61 cm and a 50 μm nominal I.D. (Spectrochrom, Stockholm, Sweden), while the second capillary column had a total length of 43 cm and a 30 μm nominal I.D. (Skandinaviska GeneTech, Kungsbacka, Sweden). Injections were performed in the hydrodynamic mode (gravity injection), with the sample vial elevated to 75 mm during the injection time of 10 s. The voltage applied was 20 kV when running the instrument in the constant voltage mode, while in the constant power mode, 1200 mW (corresponding to an applied voltage of approximately 21 kV) was applied.

2.2. Reagents

All reagents used were of analytical reagent grade. Sodium cyanoborohydride, 6-AQ, acetic acid and trifluoroacetic acid were purchased from Aldrich (Milwaukee, WI, USA). Commercially available monosaccharides and uronic acids were obtained from Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland) or from Merck (Darmstadt, Germany). 4-O-Methyl-D-glucuronic acid (4-O-Me-D-GlcA) was synthesized by Christian Krog-Jensen at the University of Stockholm (Sweden) and β-(1-4)-D-xylobiose was obtained from Sigma. The neutral [β-(1-4)-D-xylotriose, β-(1-4)-D-xylotetraose, β-(1-4)-D-xylopentaose and β-(1-4)-D-xylohexaose] and the acidic [4-O-Me-α-D-GlcA*p*-(1-2)-D-xylose, 4-O-Me-α-D-GlcA*p*-(1-2)-D-xylobiose and 4-O-Me-α-D-GlcA*p*-(1-2)-D-xylotriose] oligosaccharides were obtained from Megazyme (Sydney, Australia). For preparation of reagent and buffer solutions, ultrapure Millipore Milli-Q Plus water (Millipore, Milford, MA, USA) was employed. The running electrolyte buffers were prepared by dissolving boric acid in ultra-pure water, followed by addition of the appropriate amount of 1 mol l^{-1} sodium hydroxide to give a pH of 9. Buffer solutions containing 210–420 mmol l^{-1} H3BO3 and 110–220 mmol l^{-1} NaOH were prepared in this manner.

2.3. Derivatization of mono- and oligosaccharides

In a separate study, we used fractional factorial design techniques to assist us in finding optimal experimental conditions for the reductive amination reaction used for precolumn derivatization. This study will be descibed in detail elsewhere [25] and, therefore, only a brief description of the derivatization conditions employed will be given here.

The mono- and oligosaccharides were derivatized with 6-AQ, through reductive amination using sodium cyanoborohydride under slightly acidic conditions. A more than 30-fold excess of the derivatization reagent, 6-AQ, was used in all cases. The reaction mixtures usually contained final concentrations of 0.1 mmol l^{-1} saccharides, 30 mmol l^{-1} 6-AQ, 5 mmol l^{-1} sodium cyanoborohydride and 150 mmol l^{-1} acetic acid.

For monosaccharide analysis, it was found that a reaction time of only 45 min at a temperature of 80°C was sufficient to ensure reproducible analytical results. However, for oligosaccharide analyses, we selected a lower reaction temperature of 40°C and a reaction time of 2 h, because somewhat cleaner reaction mixtures were obtained under these conditions. After cooling to room temperature, the reaction mixtures were filtered and analyzed by CZE without prior purification.

2.4. Calibration and quantitation

An aqueous solution of L-rhamnose, D-xylose, D-glucose, D-mannose, L-arabinose, D-galactose, D-glucuronic acid and D-galacturonic acid was pretreated with 3% sulphuric acid for 40 min at 125°C. This procedure was used to simulate standard conditions used for chemical hydrolysis of wood-derived, cellulose-containing samples [10]. The resulting solution was then diluted and used to prepare a set of standard calibration solutions with con-

centrations ranging from 0.001–0.25 mmol l^{-1} for each monosaccharide, except for D-glucose (the concentration of which was 0.005–1.2 mmol l^{-1}).

To each of these calibration solutions was added the same amount of 2-deoxy-D-galactose (final concentration=3.0 mmol l^{-1}) as an internal standard. The pH of the calibration solutions was adjusted to 3.5. About 370 μl of an aqueous solution of 0.5 mol l^{-1} 6-AQ containing acetic acid (2.5 mol l^{-1}) was added, followed by the addition of 46 μl of 0.5 mol l^{-1} aqueous sodium cyanoborohydride. The solutions were subsequently incubated at 80°C for 45 min. After cooling to room temperature, the mixtures were analyzed several times (a minimum of two injections) by CZE. For each concentration, duplicates were prepared and analyzed in the same fashion.

2.5. Chemical and enzymatic hydrolysis of softwood xylan

Softwood xylan was isolated from an unbleached thermomechanical pulp according to the extraction and separation procedure described by Hansson and Hartler [26]. About 5 mg of the isolated xylan was dissolved in 1 ml of 2 mol l^{-1} trifluoroacetic acid. The resulting solution was degassed with nitrogen for 10 s, in order to remove oxygen, and thereafter was heated at 100°C for 4 h. After this hydrolysis, the sample was freeze-dried.

For the enzymatic treatment, 200 mg of xylan was solubilized in 8 ml of 50 mmol l^{-1} phosphate solution (pH 7.5) containing 200 μl of xylanase (SP473, Novo, Bagsvaerd, Denmark). The solution was incubated overnight at 40°C. The hydrolysis products were then separated from the high-molecular-mass enzyme on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden). After this separation, the hydrolyzate was freeze-dried, derivatized with 6-AQ as described in Section 2.3 and analyzed by CZE.

3. Results and discussion

3.1. Separation of monosaccharides by CZE

The separation of neutral monosaccharides by CZE requires prior conversion of these compounds into charged species. Such conversion can be accomplished by several different techniques, e.g., through the introduction of a charged functional group by precolumn derivatization or by using a buffer system capable of forming charged complexes with the hydroxyl groups of the monosaccharides. The latter alternative is especially attractive when attempting to separate wood-derived neutral monosaccharides which, in many cases, only differ from each other by the configuration of a single hydroxyl group (e.g. glucose and mannose). By using alkaline borate as the buffer solution, it has been possible to separate both underivatized [20] and derivatized [21,22,24,27,28] neutral saccharides by CZE, due to the formation of a negatively charged complex, predominantly between the tetrahydroxyborate ion and the saccharides. For monosaccharides derivatized by reductive amination (2-aminopyridine), it has been found [24] that the stability of the complex and, thus, the electrophoretic mobility, depends strongly on the configurations of the vicinal hydroxyl groups (chiefly C2, C3 and C4).

Fig. 1 shows an electropherogram of a standard mixture containing 6-AQ derivatives of eight monosaccharides analyzed at constant voltage using a 220 mmol l^{-1} alkaline borate buffer and a capillary column with an inner diameter of 50 μm. The first peak detected corresponds to the derivatization reagent 6-AQ, which is present in excess in the reaction mixture after derivatization. Under the analytical conditions used, 6-AQ behaves as a neutral compound and can thus be used as a marker for the electroosmotic flow. The monosaccharide 6-AQ derivatives, which are negatively charged under the same conditions, migrate against the electroosmotic flow and are therefore eluted after the 6-AQ peak.

Among the neutral monosaccharide 6-AQ derivatives in the electropherogram shown in Fig. 1, xylose was eluted first, followed by glucose, mannose, arabinose and galactose. The 6-AQ derivatives of the uronic acids were detected much later in the electropherogram. Compared to the neutral monosaccharides, the uronic acids have a higher charge density at the pH used, due to their negatively charged carboxyl group. These compounds are therefore transported more slowly through the capillary column (due to a higher electrophoretic mobility in the opposite direction to that of the electroosmotic

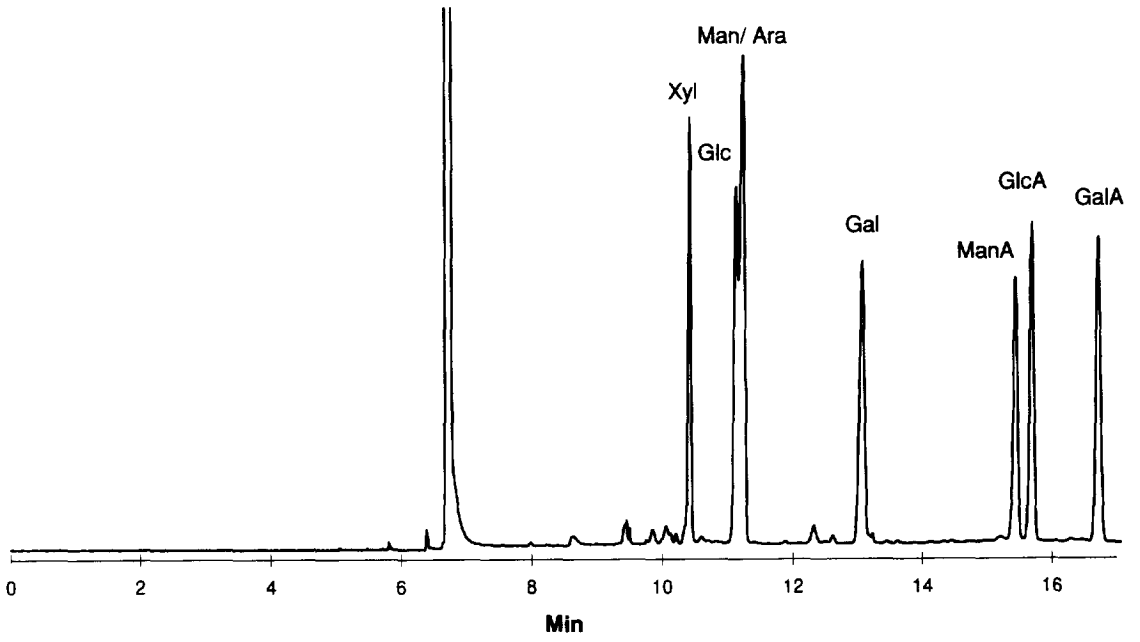

Fig. 1. CZE analysis of 6-AQ derivatives of a standard mixture of eight different monosaccharides in 220 mmol l^{-1} alkaline borate buffer, pH 9; capillary, uncoated fused-silica, L = 61 cm, l = 56 cm, I.D. = 50 μm; detection, UV absorption at 245 nm; applied voltage, 20 kV (I = 42 μA). Xyl = xylose, Ara = arabinose, Glc = glucose, Man = mannose, Gal = galactose, ManA = mannuronic acid, GlcA = glucuronic acid, GalA = galacturonic acid.

flow). The relative order of electrophoretic migration observed for many of the 6-AQ derivatives in Fig. 1 was the same as that previously reported for CZE analysis of the corresponding 2-aminopyridine (2-AP) derivatives in alkaline borate buffer [24].

Fig. 2A depicts an expanded portion of the electropherogram shown in Fig. 1. As can be seen in Fig. 2A, the 6-AQ derivatives of glucose, mannose and arabinose were only poorly resolved. The separation of carbohydrates by CZE in alkaline borate depends strongly on the relative stability of the borate complexes formed [20,24]. The mole fraction of the carbohydrate complexed with borate can be increased by increasing the pH or the borate concentration [20]. For example, Honda et al. [24] observed increased resolution upon CZE analysis of several 2-AP-derivatized monosaccharides when the borate buffer pH was increased to around 10 or 11. However, even at optimal pH, Honda et al. [24] observed incomplete separation of the 2-AP derivatives of glucose and arabinose (the 2-AP derivative of mannose was not investigated). We therefore investigated the possibility of increasing the resolution between the glucose, mannose and arabinose 6-AQ derivatives by using a more concentrated borate buffer.

In Fig. 2B, the separation of the 6-AQ derivatives of glucose, mannose and arabinose by CZE in 420 mmol l^{-1} alkaline borate buffer is shown. As can be seen, the glucose peak is now completely resolved from the mannose and arabinose peaks. The separation between mannose and arabinose was also quite good, although baseline separation was not fully achieved. Thus, the increase in borate buffer concentration and the associated increase in the mole fraction of the borate complexes resulted in a major improvement in the separation of these 6-AQ derivatives.

However, the increase in the mole fraction of the complexes also caused an increase in electrophoretic mobility, resulting in slower migration through the column. Thus, the use of the more concentrated borate buffer gave rise to an increase in the total CZE analysis time from around 16 min to slightly more than 30 min, at constant applied voltage. Furthermore, a large increase in the current flowing

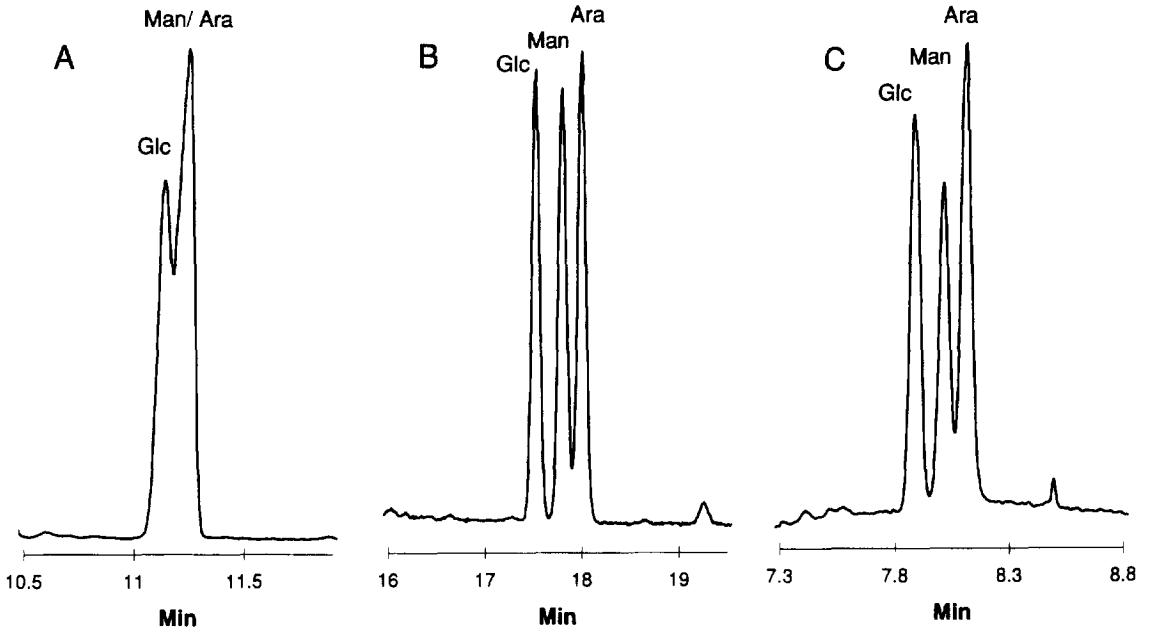

Fig. 2. CZE analysis of the 6-AQ derivatives of glucose, mannose and arabinose. (A) Expanded portion of Fig. 1. (B) Buffer, 420 mmol l^{-1} alkaline borate, pH 9; capillary, uncoated fused-silica, L = 61 cm, l = 56 cm, I.D. = 50 μm; detection, UV absorption at 245 nm; applied voltage, 20 kV (I = 81 mA). (C) Buffer, 420 mmol l^{-1} alkaline borate, pH 9; capillary, uncoated fused-silica, L = 43 cm, l = 38 cm, I.D. = 30 μm; detection, UV absorption at 245 nm; applied power, 1200 mW (I = 55 μA).

through the capillary system was observed. This increased current was most probably due to the higher ionic strength and conductivity of the more concentrated buffer and it produced increased heating (Joule heating) of the capillary. Furthermore, the reproducibility of the migration times for the 6-AQ derivatives in separate CZE analyses became very poor, probably due to the accumulation of heat in the capillary.

In order to reduce the current flowing through the capillary column and thereby overcome the problems associated with increased Joule heating, the 50 μm I.D. column was replaced by a column with a smaller inner diameter (30 μm I.D.). The current generated in a capillary column is proportional to the square of the inner radius [16,17]. Therefore, a decrease in the inner diameter of a column should lead to a dramatic decrease in current and, thus, to a decrease in Joule heating as well. In addition, the increase in the surface area-to-volume ratio should allow for more effective dissipation of the heat still generated. Furthermore, by using a shorter capillary column, the total CZE analysis time should be decreased considerably. The separation of the 6-AQ derivatives should not be effected negatively by a change to a shorter capillary column with a smaller inner diameter [15].

Fig. 2C shows an expanded portion of the electropherogram of the 6-AQ derivatives using a 420 mmol l^{-1} alkaline borate buffer and a capillary column with a length of 43 cm and an inner diameter of 30 μm. The peaks corresponding to the 6-AQ derivatives of glucose, mannose and arabinose are separated with a resolution of 1.0–1.2 or greater. In Fig. 3 the corresponding complete electropherogram is illustrated. All of the nine neutral and acidic monosaccharides are more efficiently separated by this method than by previously suggested CZE procedures. Under these conditions, the current was still quite unstable, varying by a few μA during a single analytical run, which led to poor reproducibility with respect to migration times. Therefore, the operating mode of the instrument was changed from voltage to power control in order to improve reproducibility.

The analytical characteristics of this CZE sepa-

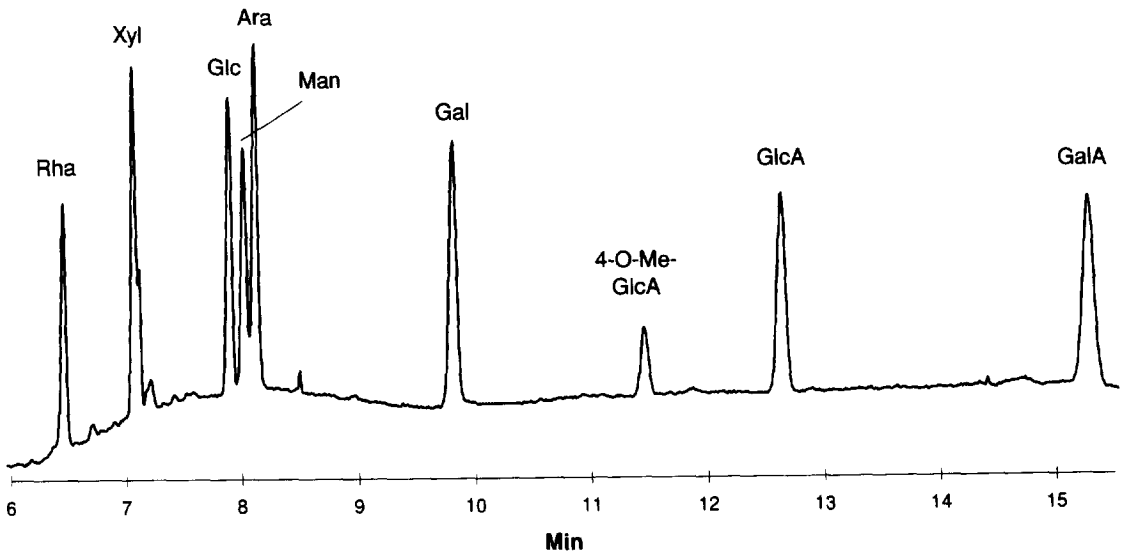

Fig. 3. CZE analysis of 6-AQ derivatives of a standard mixture of nine monosaccharides. The analytical conditions employed were as in Fig. 2C. The peaks are designated as in Fig. 1, except that Rha = rhamnose and 4-O-Me-GlcA = 4-O-Me-glucuronic acid.

ration procedure, based on four different analyses, are documented in Table 1 (with a constant power of 1200 mW). The electrophoretic mobility, μ, was calculated according to Eq. 1:

$$\mu = \frac{Ll}{V} \cdot (t^{-1} - t_{eof}^{-1}) \quad (1)$$

and the theoretical plate number was calculated using Eq. 2:

$$N = 5.54 \cdot (t/w^{1/2})^2 \quad (2)$$

as described previously [16]. In Eq. 1, L is the total length of the capillary, l is the length of the capillary from the injection site to the detector, V is the applied voltage and, finally, t and t_{eof} are the migration time for the saccharide and for the electroosmotic flow marker, respectively. The resolution, R_s, between two adjacent monosaccharide peaks was calculated from Eq. 3:

$$R_s = 2(t_1 - t_2)/(W_1 + W_2) \quad (3)$$

where t_1 and t_2 are the migration times and W_1 and

Table 1
Electrophoretic mobilities (μ), resolution between adjacent peaks (R_s) and theoretical plates (N) in the CZE analysis of the 6-AQ derivatives of nine different monosaccharides.

Monosaccharide	μ ($cm^2\ V^{-1}\ s^{-1}) \cdot 10^{-4}$	R.S.D. (%)	R_s	N ($\cdot 10^5$)
Rhamnose	−1.272	1.0		1.2
			7.0	
Xylose	−1.436	0.8		1.4
			9.4	
Glucose	−1.623	1.4		1.5
			1.2	
Mannose	−1.647	1.2		1.3
			1.0	
Arabinose	−1.666	1.2		1.5
			13.7	
Galactose	−1.917	1.5		1.0
			11.1	
4-O-Me-glucuronic acid	−2.075	1.0		1.3
			7.8	
Glucuronic acid	−2.187	1.6		1.4
			13.8	
Galacturonic acid	−2.350	1.7		1.1

R.S.D. = relative standard deviation (n = 4). Conditions: 420 mmol l^{-1} borate buffer, pH 9; a capillary column with L = 43 cm, l = 38 cm and I.D. = 30 μm and a constant power of 1200 mW (55 μA) were used.

W_2 are the baseline peak widths (in min) [16]. As can be seen in Table 1, the nine monosaccharides were all separated with R_s values ≥ 1.

3.2. Quantitation

In general, the volume of the sample introduced into the capillary column is very small (a few nanoliters). Therefore, depending on the UV–chromophoric derivatization reagent used, quite high concentrations of each monosaccharide in the sample solution are often needed. For example, Honda et al. [24] observed a linear calibration curve in the concentration range of 10–100 mmol l^{-1} for a number of 2-AP-derivatized monosaccharides.

Since saccharides labelled with 6-AQ have been reported to give much stronger UV signals than the corresponding 2-AP derivatives [23], we investigated the calibration range for our CZE procedure using more dilute (≤ 5 mmol l^{-1}) solutions of a purified 6-AQ derivative of glucuronic acid. By plotting the observed peak area (mean of two sample injections) vs. the sample concentration, a linear calibration curve was obtained in the concentration range of $1 \cdot 10^{-6}$–$2.5 \cdot 10^{-3}$ mol l^{-1}. The least-squares linear regression coefficient for this curve was $r^2 = 0.996$. Thus, the lowest detectable concentration of the 6-AQ derivative of glucuronic acid was approximately $1 \cdot 10^{-6}$ mol l^{-1}. With an injection volume of a few nanolitres, a few femtomoles was the estimated limit of detection for the 6-AQ derivative of glucuronic acid.

Calibration of our procedure, including the 6-AQ derivatization step, was performed for the eight monosaccharides listed in Table 2. A calibration curve was constructed for each monosaccharide by plotting the ratio of the amount, y, vs. the ratio of the measured peak area, x, of the 6-AQ derivative relative to the internal standard, i.e., the 6-AQ derivative of 2-deoxygalactose. Linear calibration curves were obtained in the concentration range of 5–$250 \cdot 10^{-6}$ mol l^{-1} for all saccharides, except for glucose which gave a linear curve in the range of $25 \cdot 10^{-6}$–$1.2 \cdot 10^{-3}$ mol l^{-1}.

Thus, quantitation was linear over about two orders of magnitude of concentration. In Table 2, regression equations in the form $y = k \cdot x + m$ and the regression coefficients (r^2), obtained by least-squares linear regression analysis, are given for each saccharide. In addition, the relative standard deviations for the determination of each saccharide, at concentrations of 5 and $250 \cdot 10^{-6}$ mol l^{-1}, are presented. Table 2 demonstrates that this procedure provides reproducible quantitation within the calibration range.

3.3. Separation of oligosaccharides by CZE

In kraft pulps, especially hardwood (birch) pulps, hemicelluloses of the xylan type are abundant polysaccharide constituents. Upon mild acidic [1,29] or enzymatic hydrolysis [14], pulp xylan yields both neutral and acidic oligosaccharides. Analysis of such oligosaccharides may provide useful structural in-

Table 2
Relative standard deviations (R.S.D.) of quantitation, regression equations and regression coefficients for the eight monosaccharides used in the calibration of the CZE analysis.

Monosaccharide	Quantitation R.S.D. (%)		Regression	
	$5 \cdot 10^{-6}$ mol l^{-1}	$250 \cdot 10^{-6}$ mol l^{-1}	Equation $y = kx + m$	Regression coefficient r^2
Rhamnose	2.0	4.2	$y = 1.398x - 0.017$	0.996
Xylose	0.1	1.3	$y = 1.007x - 0.007$	0.999
Glucose	4.2[a]	11.1[b]	$y = 1.997x - 0.040$	0.981
Mannose	5.3	1.7	$y = 0.984x - 0.002$	0.999
Arabinose	3.8	4.9	$y = 0.784x - 0.002$	0.996
Galactose	3.0	2.2	$y = 0.857x - 0.018$	0.999
Glucuronic acid	13.4	0.6	$y = 0.903x + 0.002$	0.999
Galacturonic acid	3.8	3.9	$y = 1.098x - 0.003$	0.995

[a] $25 \cdot 10^{-6}$ mol l^{-1}.
[b] $1.2 \cdot 10^{-3}$ mol l^{-1}.

formation about the xylan. Thus, CZE analysis of some commercially available neutral xylose oligomers, as well as of acidic xylose oligomers containing one 4-O-Me-α-D-gluco-pyranosyluronic acid unit (4-O-Me-α-D-GlcA*p*), all derivatized with 6-AQ, was performed using the optimal conditions established for the monosaccharides. The high degree of resolution of the 6-AQ derivatives of xylan-derived neutral and acidic oligosaccharides by CZE is shown in Fig. 4 and Fig. 5.

In Fig. 4, CZE analysis of a mixture of D-xylose and five β(1-4)-D-xylooligomers is shown. In this electropherogram, the xylooligomers are all well separated (baseline separation) within a separation window of less than 2 min. The largest xylooligomer, the xylohexamer, eluted first, followed by the smaller oligomers in order of decreasing size. This result is quite reasonable, since the charge density (molecular charge to size ratio) has a pronounced influence on the electrophoretic mobility [28]. The charge density of the borate–carbohydrate complex is expected to decrease as the size of the xylooligomer increases, due to less effective borate complexation with the pyranose ring form of xylose.

Illustrated in Fig. 5 is the CZE analysis of a mixture of xylan-derived acidic oligosaccharides derivatized with 6-AQ. This mixture contained 4-O-Me-D-glucuronic acid (peak 1), 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylose (peak 2), 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylobiose (two isomers, peaks 3 and 4) and 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylotriose (two major isomers, peaks 5 and 6). As can be seen, the acidic oligosaccharides are well separated within an elution time of 10 min. The isomers of 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylobiose (aldotriuronic acids) and of 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylotriose (aldotetrauronic acids) are also well separated, further illustrating the efficiency of the separation achieved by the CZE procedure employed.

3.4. CZE analysis of spruce wood xylan after chemical or enzymatic hydrolysis.

The CZE analysis of the 6-AQ derivatives of the saccharides obtained upon enzymatic (xylanase) or chemical (trifluoroacetic acid) hydrolysis of a sample of xylan hemicellulose from spruce wood are shown in Fig. 6 and Fig. 7, respectively. The saccharides in these figures were identified by spiking the samples with authentic reference compounds. The elec-

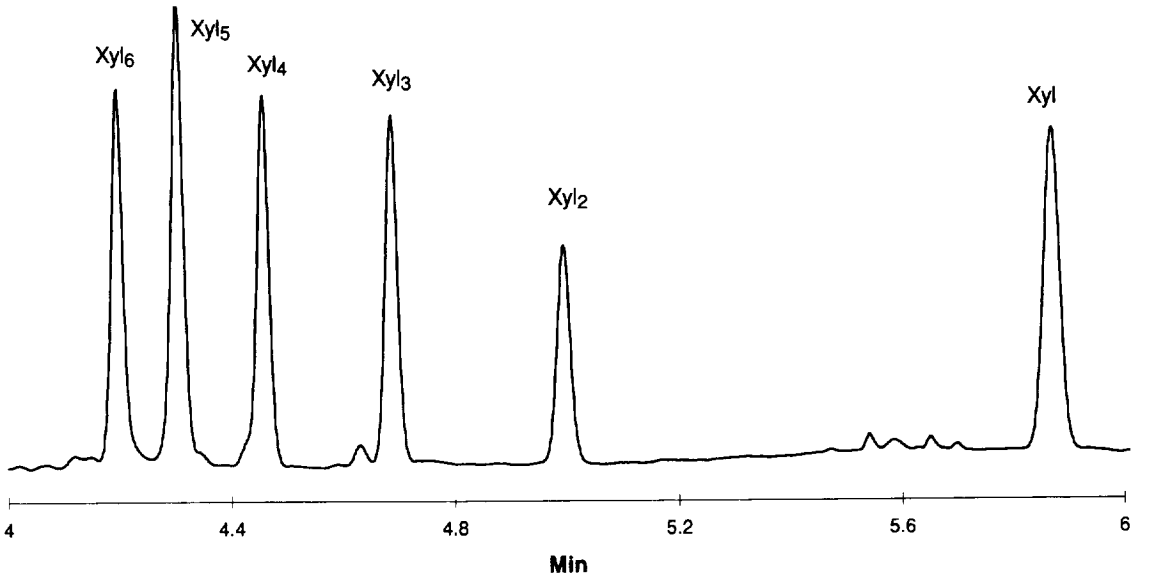

Fig. 4. CZE analysis of the 6-AQ derivatives of a standard mixture of xylose and five neutral xylooligosaccharides. Xyl = xylose, Xyl_2 = xylobiose, Xyl_3 = xylotriose, Xyl_4 = xylotetraose, Xyl_5 = xylopentaose and Xyl_6 = xylohexaose. The analytical conditions were as described in the legend to Fig. 2C, except that the voltage applied was 20 kV.

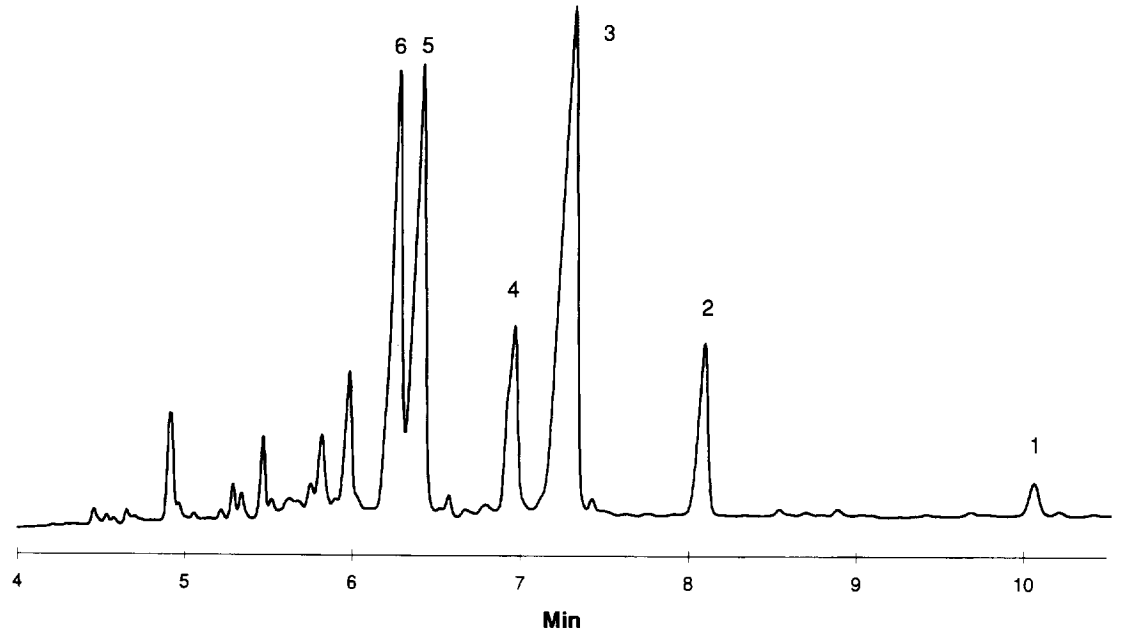

Fig. 5. CZE analysis of the 6-AQ derivatives of commercially available xylan-derived acidic oligosaccharides. Peaks: 1 = 4-O-Me-glucuronic acid, 2 = aldobiuronic acid, 3 and 4 = aldotriuronic acid (two isomeric forms), 5 and 6 = aldotetrauronic acid (two isomeric forms). The analytical conditions were as described in the legend to Fig. 2C, except that the voltage applied was 20 kV.

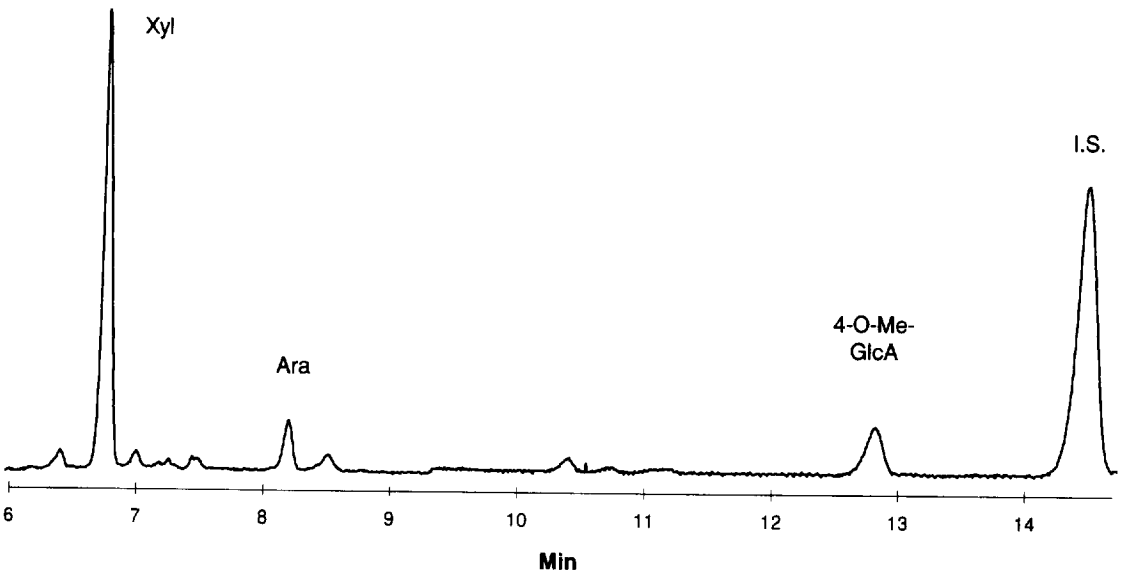

Fig. 6. CZE analysis of the 6-AQ derivatives of the products obtained by hydrolyzing a sample of spruce wood xylan with xylanase. Xyl = xylose, Ara = arabinose, 4-O-Me-GlcA = 4-O-Me-glucuronic acid, I.S. = the internal standard mannuronic acid (used for quantitation).

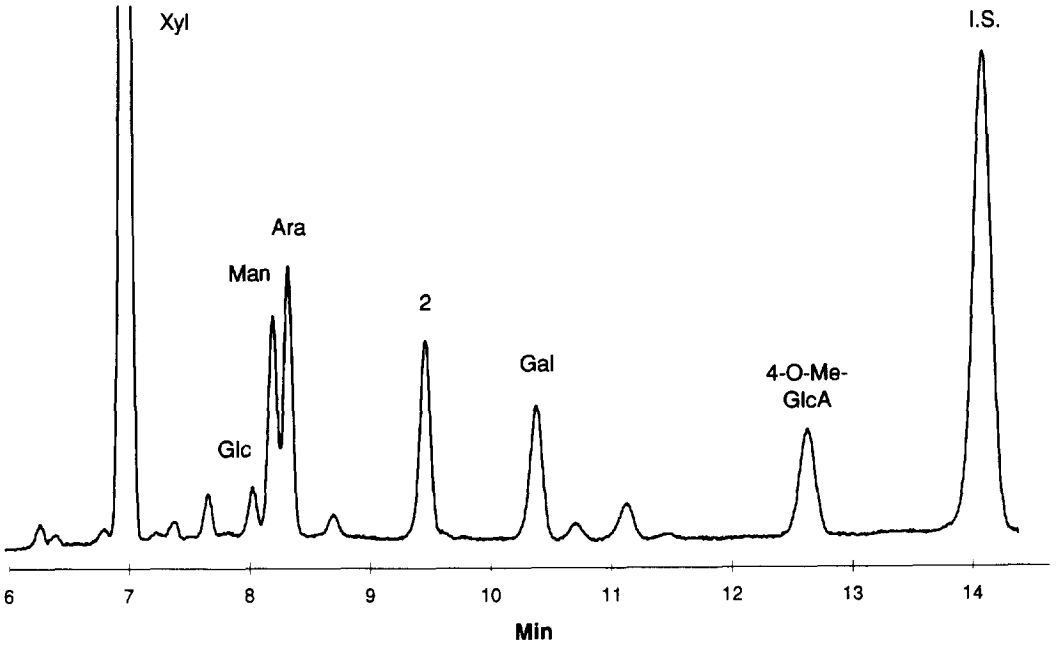

Fig. 7. CZE analysis of the 6-AQ derivatives of the products obtained by hydrolyzing a sample of a spruce wood xylan with trifluoroacetic acid. Xyl = xylose, Glc = glucose, Man = mannose, Ara = arabinose, 2 = aldobiuronic acid, Gal = galactose, 4-O-Me-GlcA = 4-O-Me- glucuronic acid, I.S. = the internal standard mannuronic acid (used for quantitation).

tropherogram shown in Fig. 6 contains a large peak corresponding to the 6-AQ derivative of D-xylose, the major monosaccharide unit in spruce wood xylan, and two minor peaks containing the derivatives of L-arabinose and 4-O-Me-D-GlcA. From the CZE analysis of the enzymatically hydrolyzed sample (Fig. 6), the relative amounts of D-xylose, L-arabinose and 4-O-Me-D-GlcA in the xylan sample were found to be 10:1:1.3, respectively. This ratio agrees well with the corresponding values commonly reported for softwood xylans [1].

CZE analysis of the chemically hydrolyzed sample showed (Fig. 7), in addition to the xylan-derived saccharides, peaks corresponding to glucose, mannose and galactose, which probably originate from the presence of galactoglucomannan as a minor impurity in the hemicellulose sample. In addition to the peaks from the monosaccharides, a peak corresponding to the 6-AQ derivative of the disaccharide 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylose was detected (peak 2 in Fig. 7). Detection of this disaccharide in the trifluoroacetic acid hydrolyzate was expected, since the glycosidic linkage between the 4-O-Me-α-D-GlcA*p* unit and the D-xylose residue is known to be quite resistant to acid hydrolysis [30]. By assuming equal molar response factors for the 6-AQ derivatives of the disaccharide 2-O-(4-O-Me-α-D-GlcA*p*)-D-xylose and D-xylose, the relative amounts of the monosaccharide units D-xylose, L-arabinose and 4-O-Me-D-GlcA*p* were estimated to be 10:1:2.1. CZE analysis of the chemically hydrolyzed sample thus indicated a somewhat higher relative amount of the 4-O-Me-α-D-GlcA*p* unit in this softwood xylan than was observed for the enzymatically hydrolyzed sample.

4. Conclusions

A rapid and simple analytical procedure, based on CZE, for the identification and quantitation of neutral and acidic mono- and oligosaccharides from wood-derived hemicelluloses has been developed. This procedure is highly sensitive with a minimal detectable concentration of approximately $1–5 \cdot 10^{-6}$ mol l^{-1}. It also gives good separation ($R_s \geq 1$) and

accurate quantitative analysis results within a linear calibration range of two orders of magnitude. When combined with prior chemical or enzymatic hydrolysis, this analytical procedure can be used as a powerful tool for the characterization of hemicelluloses obtained from, e.g., wood pulps.

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