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Characterization of totally chlorine-free effluents from Kraft pulp bleaching
II. Analysis of carbohydrate-derived constituents after acid hydrolysis by capillary zone electrophoresis

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Abstract

A capillary zone electrophoresis (CZE) method for analyzing the total acid hydrolysis products (monosaccharides and their derivatives) of the dissolved carbohydrates from a totally chlorine-free bleaching plant was developed. Several borate buffer concentrations and other running conditions were tested. Neutral monosaccharides (arabinose, galactose, glucose, mannose, rhamnose and xylose) in hydrolysates were derivatized by means of 4-aminobenzonitrile and resolved by 500 m*M* borate buffer (pH 9.5, 16.0 kV). The detection level was less than 10 fmol. In addition, the separation of some uronic acids and oligosaccharides was studied. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Kraft pulp; Monosaccharides; Uronic acid; Oligosaccharides

1. Introduction

The analysis of carbohydrate mixtures as such or after hydrolysis is important in the pulp and paper industry. Several instrumental techniques, including primarily gas–liquid chromatography (GLC), high-performance liquid chromatography (HPLC), ion chromatography (IC), thin-layer chromatography (TLC) and supercritical fluid chromatography (SFC) with various running conditions have been used for the separation of different constituents [1–9]. The most commonly applied analysis techniques are GLC and HPLC. However, capillary zone electrophoresis (CZE) has recently also become one of the most promising novel techniques for the analysis of carbohydrate components [10]. This is mainly due to the possibility of applying this method directly to aqueous samples and with a higher separation efficiency than that of HPLC [11].

In the case of carbohydrates, the important factor in CZE is to find suitable detection method. Since carbohydrates have no unsaturated structural units, the direct use of the commonly-available ultraviolet (UV) and fluorescence detectors is not possible and various derivatization procedures must be applied to carbohydrates before their separation or detection [12]. The major derivatization procedures are based on reductive amination with 4-aminobenzonitrile (4-ABN) [13], 2-aminopyridine [14], 4-aminobenzoic acid [15,16], 8-aminonaphtalene-1,3,6-trisulfonic acid [17], or 6-aminoquinoline [18]. In addition, an

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indirect UV detection based on sorbic acid or riboflavin has been performed [19].

Another difficulty arises from the fact that neutral carbohydrates have no charge, except under strong alkaline conditions. Consequently, carbohydrate molecules have no electrophoretic mobility in acidic, neutral, or mild alkaline media and can not be separated as such from each other by CZE. However, a charge can be introduced into carbohydrate molecules by derivatization or by micelle or complex formation. The most common procedure for adding a charge to carbohydrate analytes is so-called carbohydrate–borate complexation [20], but micellar electrokinetic chromatography [13] can be used as well.

The main purpose of this study was to create a simple method of analysis for the separation of wood-derived monosaccharides. It was concluded that CZE results in a higher accuracy compared to that of GLC, since in the latter case the determination error due to the integration of the multiple peaks is generated from each monosaccharide (α- and β-anomers of the pyranoside and furanoside forms). Rydlund and Dahlman [18] have reported a CZE method for wood-derived neutral and acidic mono- and oligosaccharides as 6-aminoquinoline derivatives in borate buffer. They have succeeded in separating not only neutral monosaccharides, but also 4-*O*-methylglucuronic, glucuronic and galacturonic acids. However, in that method baseline-to-baseline separation was not achieved for glucose, mannose, and arabinose. Nguyen et al. [21] have studied plant hydrolyzates as 4-aminobenzoate and 4-ABN derivatives using borate buffer and organic solvents. A good separation was achieved also for glucose, mannose, and arabinose when mixture of organic solvents was added to borate buffer. The ultimate aim of this study was to resolve all common neutral wood-derived monosaccharides after sulfuric acid hydrolysis by borate buffer without adding any organic solvent, and to use this method in characterizing carbohydrates in totally chlorine-free (TCF) bleaching effluents.

2. Experimental

2.1. Calibration of CZE

The CZE device was calibrated with the aqueous standard mixtures of neutral monosaccharides (Table 1) in the concentration range 5–80 mg/l as a nine-point calibration with the correlation coefficient between 0.991–0.995. Ribose was used as an internal standard (I.S.) and its concentration was kept constant (10 mg/l). Some uronic acids and oligosaccharides (Table 2) were also used in order to determine their migration times. All the model substances were chromatographically pure.

2.2. Effluent samples and hydrolysis

An effluent sample (E_{op} stage) was obtained from a full-scale TCF bleach plant which produces pine (*Pinus sylvestris*) Kraft pulp. The sample was fil-

Table 1
Separation of the monosaccharides in the sample E_{op} by CE and GLC (R.S.D. refers to relative standard deviation and *n* to the number of analysis)

Compound	CE				GLC		Difference, R.S.D. (%)
	Mobility [10^{-4} cm^2/(V s)]	Concentration (mg/l)	R.S.D. (%, *n*=5)	R^c	Concentration (mg/l)	R.S.D. (%, *n*=2)	
L-Rhamnose (12[a])	−1.373	–[b]	–	0.991	–[d]	–	–
D-Xylose (15)	−1.562	114.2	8.7	0.993	143.2	5.6	15.9
D-Glucose (16)	−1.738	45.1	1.5	0.992	64.9	2.9	25.5
D-Mannose (17)	−1.770	64.8	4.3	0.995	55.1	4.8	11.4
L-Arabinose (18)	−1.809	22.0	4.9	0.994	29.5	2.9	20.6
D-Galactose (19)	−2.039	49.8	3.9	0.994	45.4	3.9	6.5

[a] Indicates peak number in Fig. 2.
[b] Peak overlaps with mannobiose peak.
[c] The correlation coefficient of the calibration curves.
[d] One peak overlaps with one xylose-derived peak.

Table 2
Mobilities of the acidic monosaccharides and oligomers in the order of their migration time

Compound	Mobility $[10^{-4}\ cm^2/(V\,s)]$
Oligosaccharides	
1,4-β-D-Xylopentaose (1[a])	−0.779
1,4-β-D-Cellohexaose (2)	−0.779
1,4-β-D-Xylotetraose (3)	−0.869
1,4-β-D-Cellopentaose (4)	−0.944
1,4-β-D-Xylotriose (5)	−0.995
1,4-β-D-Cellotetraose (6)	−1.041
1,4-β-D-Xylobiose (7)	−1.163
1,4-β-D-Cellotriose (8)	−1.163
1,4-β-D-Mannopentaose (9)	−1.256
1,4-β-D-Mannotetraose (10)	−1.328
1,4-β-D-Mannotriose (11)	−1.347
1,4-β-D-Mannobiose (13)	−1.373
1,4-β-D-Cellobiose (14)	−1.428
Acidic monosaccharides	
4-*O*-Methylglucuronic acid (20)	−2.224
Glucuronic acid (21)	−2.337
Galacturonic acid (22)	−2.556

[a] Indicates the peak number in Fig. 2.

Table 3
Parameters in the CZE runs tested

System	Borate buffer		Temperature (°C)	Voltage (kV)	Current (mA)
	m*M*	pH			
1	250	9.15	40	25.0	130
2	500	9.00	20	16.0	91
3	500	9.15	20	25.0	242
4	500	9.40	40	23.5	260
5	500	9.50	20	16.0	130
6	500	10.00	20	20.0	298
7	750	9.15	40	19.0	298
8	1000	9.15	20	16.8	229
9	1000	9.15	40	16.8	298
10	1000	9.15	40	17.0	298
11	1000	9.15	40	16.5	298
12	1000	9.15	60	14.3	298
13[a]	500	9.50	20	16.0	130
14[a]	500	9.50	40	16.0	130
15[a]	500	9.50	60	16.0	130

[a] Samples have been derivatized according to Procedure 2.

trated through a 0.45-μm filter and evaporated to dryness in a vacuum.

The evaporated sample was hydrolyzed with 72% H_2SO_4 according to the TAPPI standard method T250 [22–24]. The mixture obtained was cooled down and 1 ml of aqueous solution of ribose (I.S., 0.05 mg/ml) was added to the hydrolyzate. The SO_4^{2-} ions of the sample solution were exchanged for CO_3^{2-} ions by Amberlite IRA-68 (20–50 mesh) ion-exchange resin. Before derivatization the solution was evaporated to dryness in a vacuum, resulting in the release of carbon dioxide.

2.3. Derivatization

Procedure 1: Monosaccharide test standards (Table 3, systems 1–12, concentration 10 mg/l) were derivatized with 4-ABN. In the procedure 15.7 mg 4-ABN, 2.7 mg sodium cyanoborohydride and 3 ml 3.5% acetic acid were mixed with evaporated sample in a pear-shape flask. The mixture was kept for 30 min at 116°C and was then evaporated into dryness in a vacuum. Before CZE analysis 1 ml of running buffer was added to the residue.

Procedure 2: The effluent sample and each monosaccharide standard were derivatized by adding 1 ml of 4-ABN solution [10% (w/w) 4-ABN and 10% (w/w) acetic acid in methanol], 1 ml sodium cyanoborohydride (10 mg/ml), and 1 ml water to the evaporated samples. In each case the mixture was vortexed for 3 min at room temperature to facilitate dissolution and then kept for 1 h at 100°C. The sample solution was allowed to cool down after which 2 ml water was added. All samples were filtrated through a 0.45-μm filter before the CZE analysis.

2.4. CZE analysis

The CZE analyses were performed on a Hewlett-Packard 3D capillary electrophoresis device, which was equipped with a HP extended light path fused silica capillary [48.5 cm (effective length 40 cm)×50 μm I.D.]. Detection was carried out by on-column measurement of UV absorption at 286 nm with a 4-nm bandwidth. The sample was loaded by applying a pressure of 50 mbar for 10 s. Analyses were carried out using the running parameters shown in Table 3. Before each run the fused-silica capillary was flushed with 0.1 *M* NaOH for 3 min and then with water for 2 min. Finally, the capillary was equilibrated with running buffer for 3 min.

3. Results and discussion

Fifteen systems with different borate buffer concentrations, pH values, running temperatures and voltages were tested (Table 3). Four of these systems (5, 8, 9 and 13) were found to totally resolve peaks of neutral monosaccharides (Fig. 1). In practice, 1000 m*M* borate buffer forms crystals and could not be routinely used. However, no crystallization was observed in the 500 m*M* properly prepared borate buffer solution during a storage of one year at ambient temperature.

To resolve monosaccharides from each other the pH of the 500 m*M* borate buffer had to adjust to a higher level (9.5) than that of the 1000 m*M* buffer (9.15). In this system, the resolution between the peaks of monosaccharides studied was more than 1.9 (Figs. 1 and 2). Rhamnose was exception since it was overlapped with mannobiose. This result indicated that normally poorly separated monosaccharides (glucose, mannose and arabinose) can be resolved to baseline without any addition of organic solvent to the borate buffer as Nguyen et al. [21] have proposed.

The final selection of the two derivatization procedures tested was based on the study of Schwaiger et al. [13]. When a methanol–water mixture was used instead of the borate buffer to dissolve the sample after derivatization, migration times were reduced (Table 3, systems 5 and 13). It should be pointed out that in this case the addition of methanol in the running buffer would increase migration times due to a decrease in the electroosmotic flow. However, the methanol–water mixture in the sample decreased the conductivity of the sample. Therefore, a relatively higher field will be developed across the sample zone

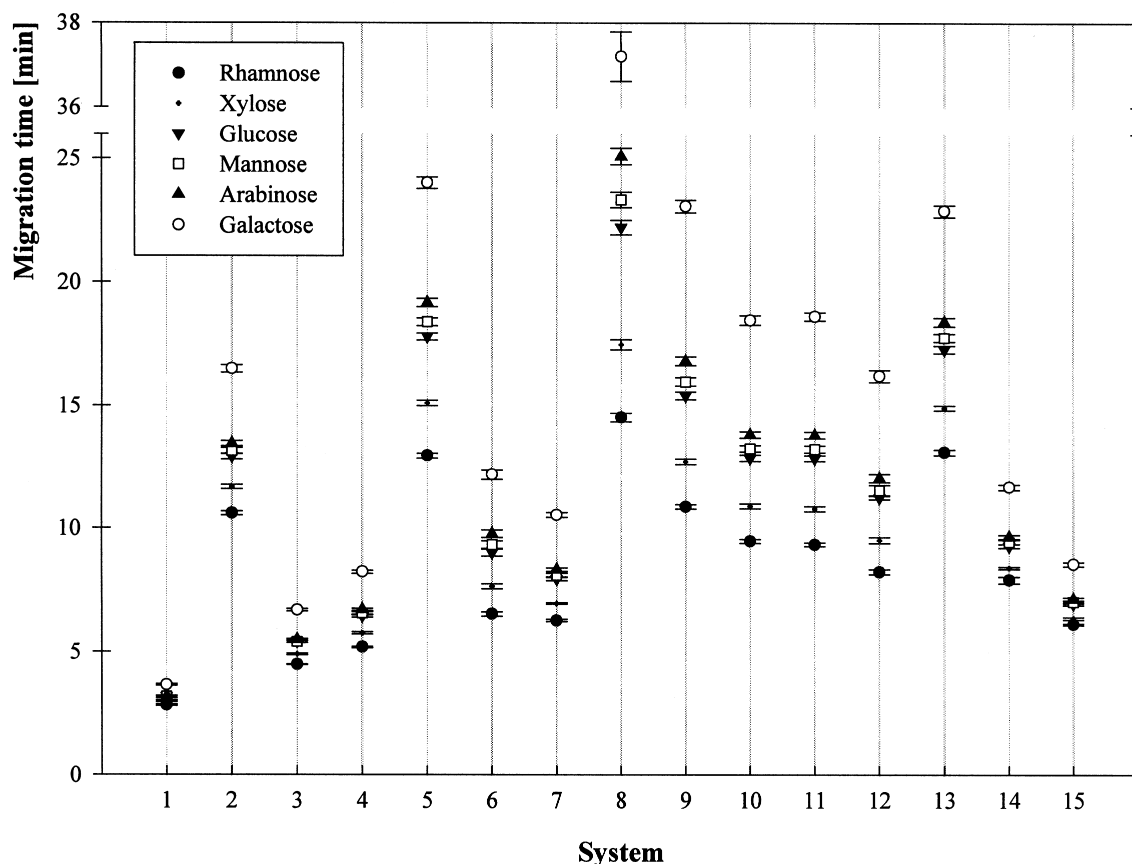

Fig. 1. The migration times for the monosaccharides studied (peaks half-width marked as ———).

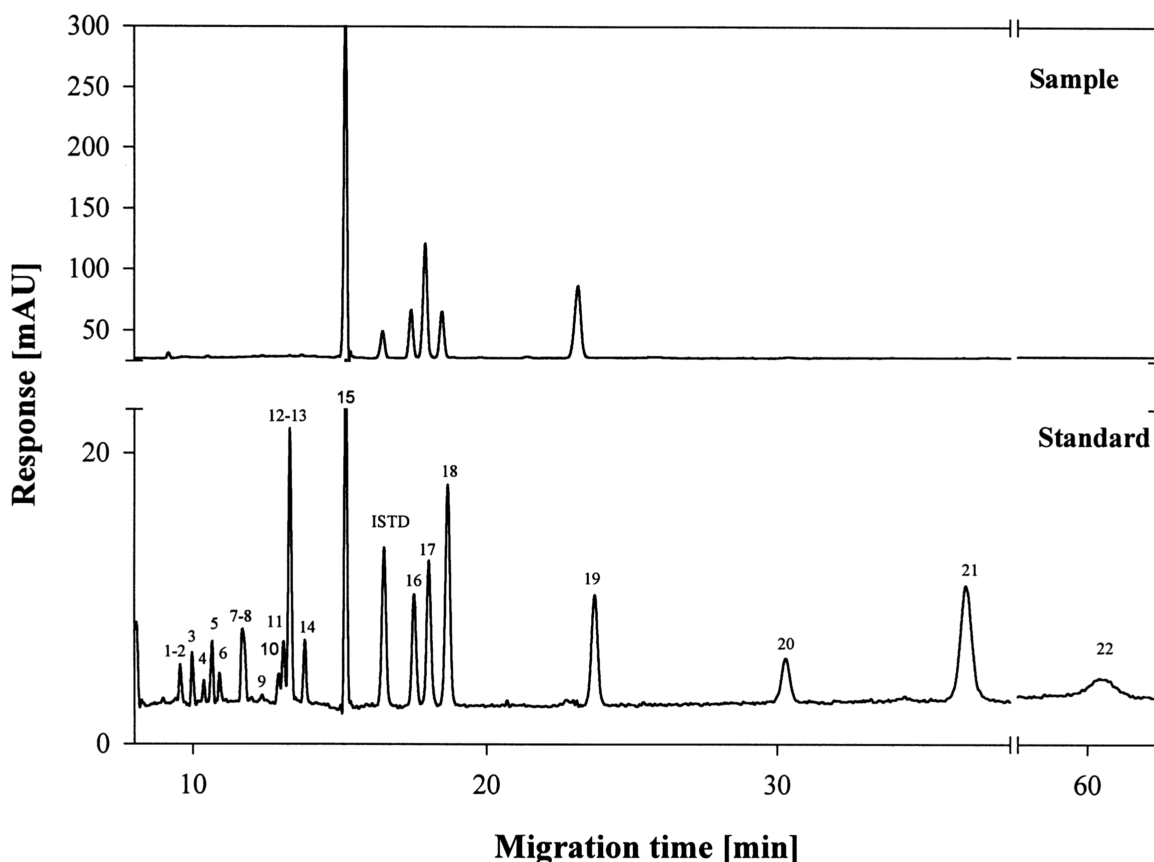

Fig. 2. The electropherograms of the standard carbohydrate solution and the hydrolysate from the sample (E_{op}). For the peak explanation, see Tables 1 and 2.

causing the analytes to migrate faster. The detection limit for neutral monosaccharides at 286 nm wavelength was 0.5 mg/l, which corresponded to less than 10 fmol of the injected amount.

At elevated temperatures (40°C and 60°C) the peak widths were considerably diminished with shortened migration times and a reduced resolution. The finding of a reduced resolution disagrees with the earlier results [11,21]. The main reason for the weak resolution at elevated temperatures was considered to be an increased electroosmotic flow due to a strongly temperature-dependent electrical field strength in high buffer concentrations [25,26]. Therefore, the lowest temperature (20°C) was selected for the CZE analysis.

The effluent of the alkaline extraction stage reinforced with oxygen and peroxide (E_{op}) was selected as a reference sample. CZE results with relative standard deviations (R.S.D.s) are compared (Table 1) to those obtained by GLC [22]. In case of GLC, higher concentrations of arabinose, glucose and xylose and lower concentrations of galactose and mannose were detected than those obtained by CE. The R.S.D. in the determination of the total amounts of monosaccharides was 8.8% and varied for individual components in the range 6.5–25.5% between these two methods. The largest R.S.D.s were observed for arabinose (20.6%) and glucose (25.5%). The R.S.D.s varied between the repeated samples in the range 1.5–8.7% (CE) and 2.9–5.6% (GLC). In both methods, the highest R.S.D. was obtained for xylose (5.6–8.7%). The deviations of the other monosaccharides were less than 5%. It should be pointed out that the R.S.D. also includes

the errors caused by incomplete formation of hydrolysis products and sample preparation.

The separation of rhamnose was difficult with both of the methods of analysis (CZE and GLC) studied. One of the rhamnose peaks overlapped with one xylose-derived peak in the GLC chromatogram, but the resolved peaks can be used to verify the presence of rhamnose. Incomplete hydrolysis caused problems in CZE, since the rhamnose peak overlapped with mannobiose. Neither mannobiose nor rhamnose were found, but traces of cellobiose and xylotriose were detected (Fig. 2). This indicated that the acid hydrolysis of the polysaccharides in our sample remained incomplete in the method applied. Therefore, a CZE system can be also used for monitoring whether hydrolysis has in fact cleaved all the glycosidic bonds in the carbohydrate-derived material. Since some oligomer peaks overlapped with each other (xylopentaose with cellohexaose and xylobiose with cellotriose), the current system can not be applied to a quantitative determination of these oligomers. Arabinose oligomers were not investigated as these are not contained in the fraction of hemicelluloses. In addition, galactose oligomers were not included in this investigation since only an insignificant amount of arabinogalactan is found in normal stem wood of softwood and hardwood species [27].

The current CZE system made also possible the analysis of uronic acids (Table 2) such as galacturonic acid (migration time 60.4 min), glucuronic acid (36.5 min), and 4-*O*-methylglucuronic acid (30.3 min). However, due to their low stability against 72% sulfuric acid, uronic acids could not be detected after sulfuric acid hydrolysis. Therefore, other hydrolysis methods such as enzymatic or trifluoroacetic acid hydrolysis have been used [16,18,21]. In addition, preliminary results, not reported in detail in this paper, indicated that the chemical composition of the hydrolyzates from enzymatic or trifluoroacetic acid treatments can also be determined by the current CZE system.

4. Conclusions

The present CZE system provides a useful tool for analyzing the wood-derived neutral and acidic monosaccharide residues after a 4-ABN derivatization. For each compound, detection levels are lower than 10 fmol and the R.S.D.s of the repeated samples are in the range 1.5–8.7%. The system can be easily utilized, especially in applications where the composition of different hydrolysates from hemicellulose-containing bleaching effluents is needed.

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