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Determination of carbohydrates in wood, pulp and process liquor samples by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

A rapid and sensitive method has been developed for the determination of monosaccharides present in the sulphuric acid hydrolyzates of wood, pulp, magnesium-based spent sulphite liquor, kraft weak black liquor and newsprint papermachine white water. Hydrolyzates were purified using an off-line, solid-phase extraction technique, employing cross-linked N-polyvinylpyrrolidone and strong anion-exchange resin. High-performance anion-exchange chromatography with pulsed amperometric detection was used to separate and quantify wood monosaccharides present in the purified hydrolyzates. Monosaccharide results obtained by ion and gas chromatographic methods were in good agreement for all samples examined. The effect of acid concentration on monosaccharide yields from the hydrolysis of process liquors was also investigated.

1. Introduction

The determination of carbohydrates in wood, pulp and process liquors and effluents is of particular significance to paper makers and researchers in the pulp and paper industry. One of the prime objectives of any pulping process is to minimize the degradation of polysaccharides. Consequently, it is important to have an accurate and sensitive method for quantifying wood carbohydrates in a wide variety of complex matrices, in order to determine the effect of various pulping and bleaching processes. In addition, sensitive methods capable of analyzing relatively low levels of carbohydrates in samples such as white water may be required in order to assess the impact of closure as mills move to reduce the use of fresh water. Over the years, a number of chromatographic methods have been developed to meet these needs. Older techniques, such as the classic paper chromatographic method of Saeman *et al.* [1] are no longer widely used, and have largely been replaced by gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods. However, many of the GC and HPLC methods suffer from some serious limitations in terms of sensitivity, selectivity, ease of use and applicability.

GC methods require that the monosaccharides be converted to volatile derivatives such as alditol acetates [2–4] and trimethylsilyl (TMS) ethers [5–7] prior to analysis. Many of the derivatization procedures are complex and time consuming.

A number of HPLC methods utilizing low-efficiency anion-exchange [8–10], borate-complex ion-exchange [11,12], ligand-exchange and amino [13–22] columns to separate mono- and

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oligosaccharides, followed by refractive index (RI), ultraviolet (UV), colorimetric, fluorescence, enzymatic and fixed potential electrochemical detection have been reported. However, these methods often utilize columns which afford poor resolution, have narrow operating pH ranges, exhibit poor stability, require high operating temperatures and are easily fouled by sample matrix contaminants. Moreover, RI and UV detectors exhibit low sensitivity and specificity of response toward carbohydrates.

High-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD) is a relatively new technique and offers a powerful alternative to traditional HPLC methods. Ion-exchange columns used in HPAEC are polymer-based, can tolerate eluents ranging in pH from 0 to 14, and can therefore be cleaned with strong acids and bases. In addition, the PAD response is reported to be linear over four orders of sugar concentration [23] with monosaccharide levels in the 10–100 pmol range routinely detectable [24]. Moreover, PAD is about two orders of magnitude more sensitive for these analytes than UV or RI detectors [25], and is to a great extent free from interferences.

The application of HPAEC–PAD for the determination of carbohydrates was first reported by Rocklin and Pohl [26]. Over the last decade, a number of additional publications describing the use of HPAEC–PAD for the determination of mono- and polysaccharides in the acid and enzymatic hydrolyzates of wood and/or pulp [27,28]; lignocellulose [29]; soil, manure and biomass [25,30–33]; marine and aquatic plants [34]; complex biological samples [35–37]; food products [38–41]; and fibrous substrates [42,43] have appeared. In addition, comprehensive reviews of recent developments in the chromatographic analysis of carbohydrates by HPLC [44] and HPAEC–PAD [45,46] have been published.

One of the major problems associated with analyzing carbohydrates in pulp and paper samples using any LC technique is severe column fouling and degradation that can occur as a result of the high concentration of sample contaminants co-injected onto HPLC or HPAEC columns. The acid hydrolyzates of wood and pulp are relatively free from contaminants and pose little threat to the LC column. However, process liquors (such as kraft weak black and spent sulphite liquors) contain high concentrations of lignin, carboxylic/sulphonic acids, humic substances and lower levels of other wood extractives that can adsorb irreversibly on the stationary phase of a HPLC column [16]. Since these samples are so difficult to analyze, it is not surprising to find that no publication to date has described the application of HPAEC–PAD for the determination of carbohydrates in pulp and paper process liquors. This report describes the development of a simple and relatively inexpensive off-line, mixed-mode solid-phase extraction (SPE) procedure which effectively removes contaminants from wood and pulp acid hydrolyzates, and both unhydrolyzed and acid hydrolyzed process liquors, prior to carbohydrate analysis using HPAEC–PAD. A comparison of carbohydrate results obtained for a number of samples using HPAEC–PAD and GC methods is presented. The effect of sulphuric acid concentration on the yield of monosaccharides from the hydrolysis of polysaccharides present in various process liquors has also been investigated.

2. Experimental

2.1. Wood and pulp samples

The following solid samples were studied: wood samples of aspen (*Populus deltoides*) and pine (*Pinus radiata*); unbleached kraft pulp and a chlorite-delignified thermomechanical pulp (TMP). Wood samples were extracted with ethanol to remove extractives and then ground in a Wiley mill to pass a 0.4-mm screen. Air-dried samples were subjected to a two-step (primary and secondary) hydrolysis procedure similar to that used by Borchardt and Piper [2] (in the current work, primary hydrolysis time was 30 rather than 60 min as described [2]). Sample moisture content was determined by thoroughly drying a small portion of each sample at 105°C in a hot-air oven. At the end of the hydrolysis period, the samples were cooled to room temperature and neutralized to either pH 5–6 with

concentrated NH_4OH (GC analysis) or pH 6–7 with NaOH (HPAEC–PAD analysis). The neutralized samples were then transferred to 100-ml volumetric flasks and diluted to volume with water. All samples were refrigerated until sample clean-up and analysis were performed.

2.2. Process liquors

Magnesium-based spent sulphite liquor (Mg-SSL), kraft weak black liquor (WBL) and newsprint papermachine white water (PMWW) samples were obtained either from Paprican's pilot plant or from various Canadian pulp and paper mills. Liquors were subjected to secondary hydrolysis only. Typically, 10 ml of spent pulping liquor and 20 ml of white water were mixed with 1 ml and 2 ml of 72% (w/w) H_2SO_4, respectively, and sufficient water was added to give a total volume of 29 ml. Liquors were then hydrolyzed, neutralized and diluted to volume (50 ml) as described in Section 2.1 for solid sample hydrolyzates.

2.3. Reagents

All monosaccharides were obtained from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). Aqueous stock monosaccharide standards (1000 mg/l) were prepared weekly and stored at 4°C. Dilute standards (0.5–150 mg/l) were prepared daily. NaOH solution (50%, w/w) was purchased from ACP (Montreal, Canada) and was used to prepare eluents (3, 100 and 350 m*M* NaOH) and post-column reagent (350 m*M* NaOH). Deionized water was sparged for 30 min with nitrogen to remove dissolved carbon dioxide prior to use in chromatography and/or preparation of dilute NaOH solutions. Sulphuric acid (96%, w/w) and ammonium hydroxide (28%, w/w) were purchased from Anachemia (Montreal, Canada).

2.4. Solid-phase extraction

The following SPE sorbents were evaluated (approximately 300 mg of each sorbent were contained in a 3-ml polypropylene tube equipped with 20-μm polyethylene frits, unless specified otherwise): Supelclean LC-SAX, strong anion-exchange resin (3-quaternary aminopropyl, chloride form); LC-SCX, strong cation-exchange resin (3-propylsulphonic acid, hydrogen form), were purchased from Supelco (Bellefonte, PA, USA). In addition, SAX resin, SPE tubes and frits were purchased as separate items from the same supplier to allow for the preparation of custom-packed SPE tubes. Cross-linked N-polyvinylpyrrolidone (PVP), was obtained from Aldrich. Chromsep, octadecylsilane (ODS) cartridges containing 200 mg of packing in 4-ml polypropylene tubes were obtained from Chromatographic Specialties (Brockville, Canada).

Custom-packed SPE tubes containing PVP/SAX were prepared by first placing a polyethylene frit in an empty SPE tube followed by about 300 mg of SAX, 150–200 mg of PVP, and finally another frit to retain the sorbents. Each SPE tube was then conditioned with 5 ml of water (pH 7) in order to remove any contaminants from the resin which might interfere with the subsequent carbohydrate analysis. This washing step also serves to wet the resin and adjust its pH, prior to sample application.

Prior to analysis of samples using HPAEC–PAD, a 1-ml aliquot of the neutralized and diluted hydrolyzate or process liquor was transferred to either a 10-ml (white water samples) or 25-ml (spent pulping liquors) volumetric flask containing sufficient internal standard (I.S., 2-deoxy-D-glucose) to give a final I.S. concentration of 5 mg/l. A portion of the diluted sample (*ca.* 5 ml) was then transferred to a 10-ml syringe which was attached to a conditioned SPE tube. The sample was forced through the SPE tube packing at a rate of 1–2 ml/min, and the last 3 ml of eluate were retained for carbohydrate analysis.

2.5. High-performance anion-exchange chromatography

The HPAEC–PAD analyses were performed on a Dionex (Sunnyvale, CA, USA) 4000i ion chromatograph equipped with a quaternary gradient pump module (GPM). Samples were injected via a Dionex high-pressure injection valve equipped with a 100-μl sample loop. Monosac-

charides were separated on Dionex CarboPac PA guard (25 × 4 mm) and PA-1 analytical (250 × 4 mm) columns at a flow-rate of 1.0 ml/min, at ambient temperature (*ca.* 25°C). A 5-μm filter was attached to the inlet of the guard column to prevent particulate plugging of the columns. The eluents used in this work and their relative proportions are shown in Table 1. Eluent 1 was nitrogen sparged, deionized water, eluent 2 was 100 m*M* NaOH, eluent 3 was 350 m*M* NaOH.

After post-run flushing for 10 min with eluent 3, the column was equilibrated with the initial mobile phase for 10 min prior to the next sample injection. All mobile phases were stored under nitrogen to prevent absorption of atmospheric CO_2. Carbonate will act as a displacing ion and shorten retention times. Sodium hydroxide (350 m*M*) was added to the eluent stream, post-column at a rate of 0.8 ml/min via a low-dead-volume plastic mixing tee. Carbohydrates were detected using a Dionex Model PAD-1 detector equipped with gold working and silver reference electrodes (Ag electrode was filled with 350 m*M* NaOH), operating with the following working electrode pulse potentials and durations: $E_1 = +0.05$ V ($t_1 = 300$ ms), $E_2 = +0.60$ V ($t_2 = 120$ ms), $E_3 = -0.80$ V ($t_3 = 300$ ms). The PAD response time was set to 1 s and the range was 10 μA full scale. Chromatographic data were collected and plotted using a Hewlett-Packard (Avondale, PA, USA) Model 3390A integrator.

2.6. Gas chromatography

For GC analysis, a 200-μl aliquot of the neutralized secondary hydrolyzate (pH 5–6) was reduced, acetylated and extracted into CH_2Cl_2 according to the method of Harris *et al.* [4]. The monosaccharides derivatives (alditol acetates) were stored at 4°C until GC analysis was performed. Monosaccharide derivatives were chromatographed on a Hewlett-Packard 5890A gas chromatograph equipped with a DB-225 capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA) and a flame ionization detector under the following conditions: high-purity helium was used as carrier and detector make-up gas; carrier gas flow-rate was 1.5 ml/min; injector and detector temperatures were 250°C; oven temperature program, 220 to 235°C at 1°C/min, hold 2 min; injection mode, split injection (20:1). Total run time was 17 min and chromatographic data were collected and plotted using a Hewlett-Packard Model 3396A integrator.

Table 1
Eluents used to separate wood monosaccharides using HPAEC

Eluent	Eluent proportions (%, v/v)		
	0–40 min	40.1–50 min	50.1 min
1	97	0	97
2	3	0	3
3	0	100	0

3. Results and discussion

3.1. Optimization of HPAEC–PAD conditions

In the initial phase of our investigation, we were concerned with optimizing eluent composition, flow-rate and column flushing conditions in order to achieve maximum separation of the five main wood monosaccharides and internal standard, while minimizing analysis time and variations in retention time. Very good separation of all monosaccharides was obtained using 3 m*M* NaOH delivered at 1 ml/min, as indicated in Fig. 1. In previous work carried out in our laboratory [47], it was found that monosaccharide retention time decreased and peak area increased with each consecutive injection of standards. The variation in retention time was attributed to carbonate build-up during the run, but no explanation was offered for the peak area variability. Carbonate, along with acetate, nitrate and sulphate have been shown to have a high affinity for the anion-exchange column, easily displacing hydroxide ion. A high level of sulphate is present in the sulphuric acid hydrolyzates studied, moreover acetate is present in wood, pulp and process liquor hydrolyzates in

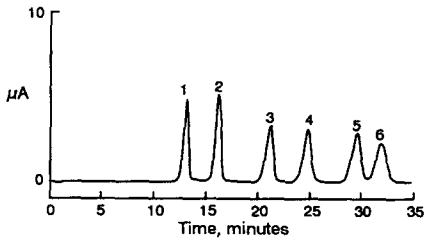

Fig. 1. Typical HPAEC–PAD chromatogram obtained for a standard mixture of wood monosaccharides. Each component present at 5 mg/l. Peaks: 1 = 2-deoxyglucose; 2 = arabinose; 3 = galactose; 4 = glucose; 5 = xylose; 6 = mannose. For analytical conditions, see Experimental section.

significant amounts. In order to obtain reproducible retention times, it is therefore essential that a post-run flush of the column with a strong NaOH solution be performed to wash strongly retained species. A 10-min post-run flush of the column with 350 m*M* NaOH, followed by a 10-min equilibration with the initial mobile phase was found to give satisfactory results. An insufficient column equilibration period could result in shorter or variable retention time because of elevated levels of OH^- remaining on the column.

The stability of the column and detector under the optimized chromatographic conditions was assessed by carrying out replicate analyses of a standard wood monosaccharide solution. As indicated in Table 2, highly reproducible retention times and relative peak areas were obtained for each monosaccharide studied.

Table 3
Linear regression data for peak area *versus* wood monosaccharide plot

Monosaccharide	Intercept	Slope	Correlation coefficient (R)
Arabinose	−0.29	1.87	0.999
Galactose	0.041	1.56	0.999
Glucose	0.11	1.75	0.999
Xylose	−0.021	1.79	0.999
Mannose	−0.078	1.60	0.999

3.2. Linearity of detector response

The detector response (peak area) for a given monosaccharide must be linear over a large concentration range in order to analyze samples containing disparate levels of monosaccharides in one chromatographic run. An example of such a sample is kraft pulp hydrolyzate, which may contain up to 150 mg/l of glucose and only 1–2 mg/l of arabinose or galactose. As shown in Table 3, the PAD response for each monosaccharide was linear over the concentration range (0.5–150 mg/l) studied, with correlation coefficients of 0.999 or better for each analyte.

In this work, 2-deoxy-D-glucose is added to hydrolyzates and used to quantify monosaccharides. Consequently, it is also essential that the relative response factor (RRF) of each monosaccharide with respect to the internal standard be linear over a wide concentration

Table 2
Repeatability (n = 7) of retention time and peak area, obtained for a standard wood monosaccharide solution containing 10 mg/l of each wood monosaccharide and 5 mg/l of internal standard

Monosaccharide	Retention time (min)		Relative peak area	
	Mean	R.S.D. (%)[a]	Mean	R.S.D. (%)
2-Deoxy-D-glucose	12.8	1.4	7.1	1.9
Arabinose	15.7	1.5	18.7	1.5
Galactose	20.8	1.5	15.8	1.5
Glucose	24.6	1.5	17.3	1.5
Xylose	29.2	1.5	17.8	0.9
Mannose	31.6	1.6	15.4	0.9

[a] R.S.D. = Relative standard deviation.

range, since accurately known RRFs are integral to the accurate determination of monosaccharide levels. The RRF of a given monosaccharide is given by Eq. 1.

$$RRF_S = \frac{A_{I.S.}}{A_S} \cdot \frac{C_S}{C_{I.S.}} \quad (1)$$

where $A_{I.S.}$ and A_S are the areas of the internal standard and monosaccharide peaks, respectively; $C_{I.S.}$ and C_S are the concentrations of the internal standard and monosaccharides, respectively. Eq. 1 is easily rearranged to give Eq. 2, a convenient form for determining RRF linearity.

$$\frac{A_{I.S.}}{A_S} = RRF_S \cdot \frac{C_{I.S.}}{C_S} \quad (2)$$

A plot of $A_{I.S.}/A_S$ *versus* $C_{I.S.}/C_S$ was prepared for each monosaccharide, over the concentration range 0.5–150 mg/l, with internal standard present at 5 mg/l in each case. Linear regression analysis of the data showed that the RRF of each wood monosaccharide was linear over the concentration range, with a correlation coefficient of at least 0.999 in each case. The RRFs for the five wood monosaccharides, obtained from the plot of Eq. 2 were: arabinose 0.78, galactose 0.93, glucose 0.82, xylose 0.80, mannose 0.92.

3.3. Evaluation of solid-phase extraction sorbents

Several publications describing the use of commercially available SPE sorbents, such as SAX, SCX, diol, cyano and amino-functionalized silica, for off-line sample clean-up of unhydrolyzed and acid and enzymatic hydrolyzates of various substrates, prior to carbohydrate analysis, have recently appeared [25,30,31,32,41]. In addition, an on-line sample clean-up scheme has recently been described by Marko-Varga and co-workers [13,16], in which three small columns containing SAX, amino-functionalized silica along with a non-silica-based hydrophobic polymer are used to purify fermentation broths. However, the effectiveness of these sorbents for sample cleanup have been quantified by only a few of these workers. Both Marko-Varga and co-workers [13,16], and Martens and Frankenberger [25,30,31], have demonstrated that SAX is very effective at removing coloured compounds from various sample hydrolyzates. In addition, Bio-Gel P-2 (Bio-Rad), a polyacrylamide gel with a low exclusion limit, also has been reported to be effective for this purpose [25]. Consequently, we assessed the potential of various SPE sorbents by determining the extent to which the five principal wood monosaccharides and internal standard could be recovered from aqueous solutions. Two standard monosaccharide solutions containing low (2–10 mg/l) and high (15–150 mg/l) levels of each component were used for this purpose. The eluates from the sorbents were analyzed for carbohydrate content and the results compared to those obtained with samples not passed through sorbents. All of the sorbents examined retained less than 5% of each monosaccharide, indicating that they were all potentially useful for sample clean-up.

The relative effectiveness of sorbents in removing sample contaminants from spent sulphite and weak black liquors were then determined. Wood and pulp hydrolyzates generally contain significantly lower levels of contaminants and were not included in this evaluation.

Magnesium-based spent sulphite liquor

Lignosulphonic acids comprise about half of the total dissolved organic matter present in spent sulphite liquor [48]. The non-carbohydrate matrix components present in this type of sample cause major problems in saccharide determination, as demonstrated in a previous study [47], and illustrated in Fig. 2. It is evident from this chromatogram that non-carbohydrate components have adsorbed onto the stationary phase of the column, severely reducing its efficiency, and resulting in co-elution of these contaminants with the monosaccharides. Extensive column flushing with strong NaOH eluent was required to restore column performance.

The relative effectiveness of various sorbents was assessed by recording the UV–Vis absorption spectrum (200–500 nm) of a neutralized (pH

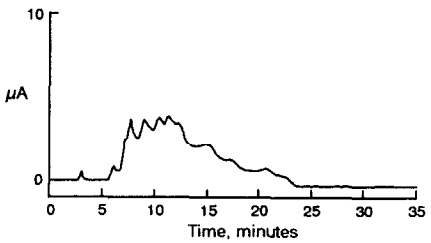

Fig. 2. HPAEC–PAD chromatogram of an acid hydrolyzate of Mg-SSL (sample from Paprican's pulping pilot plant) without sample clean-up [47].

6.5) and diluted (1:100, v/v) sample of Mg-SSL, before and after SPE. The absorption spectra of the original Mg-SSL and of samples after treatment with SPE sorbents are shown in Fig. 3A. The original Mg-SSL sample absorbs strongly from about 400 nm and below. The same liquor treated with ODS and SCX showed only a small decrease in absorption, and the eluate was still highly colored, indicating that these sorbents were only marginally effective at removing humic substances, and therefore, are not useful sorbents for this application. Samples treated with PVP and SAX showed a marked reduction in absorbance in the UV region, and both were significantly less colored. The final SPE sorbent tested was a hybrid sorbent, consisting of PVP–SAX [*ca.* 1:2 (w/w)]. This sorbent combination effectively removed a large proportion of the unwanted compounds from the sample, which absorb in the UV–Vis region. A small peak, with a maximum at about 278 nm in the spectrum of the PVP–SAX-treated sample is probably due to the absorption of low levels of lignosulphonic acids still remaining after sample clean-up. The order of effectiveness of the SPE sorbents is ODS < SCX ≪ PVP < SAX ≪ PVP–SAX.

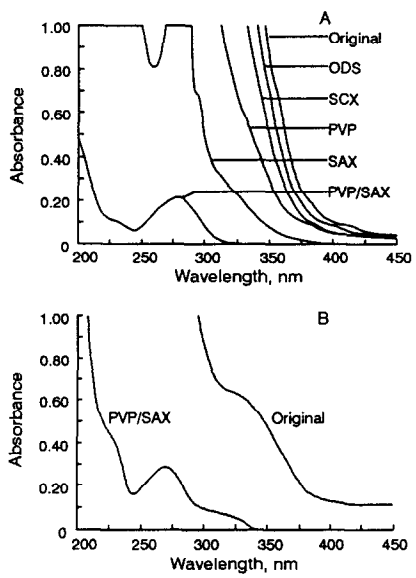

Fig. 3. The absorption spectra (200–500 nm) of an unhydrolyzed Mg-SSL (A) and an acid hydrolyzate of WBL (B), before and after SPE treatments with various sorbents (ODS = octadecylsilane; SCX = strong cation-exchange resin; PVP = cross-linked N-polyvinylpyrrolidone; SAX = strong anion-exchange resin). The pH of the Mg-SSL and WBL hydrolyzate was adjusted to 6.5 with NaOH, and diluted 1:100 and 1:125, respectively, prior to SPE. Spectra for the Mg-SSL and WBL samples were recorded with a Perkin-Elmer Model lambda 3B and a HP Model 8452A UV–Vis spectrophotometers, respectively. Water was used as reference in both cases.

Sanderson and Perera [49] have demonstrated that cross-linked PVP is extremely effective at removing polyphenolic and catechin type compounds from plant extracts, without affecting the saccharides present in the sample. More recently, reports of its use as a chromatographic sorbent for the fractionation of humic material [50] and the separation of aromatic acids, aldehydes and phenols [51] have appeared. The sorbent forms hydrogen bonds with phenolic hydroxyl and carboxyl groups, with the strength of the sorbent–phenol binding depending primarily on the number of these groups present in the molecule [51].

The mechanism by which SAX removes contaminants such as lignosulphonic acids from samples involves ion exchange. Lignosulphonic acids ($R–SO_3H$) are strong acids, and are therefore completely dissociated at pH 6–7, existing as anions (*e.g.*, lignosulphonates, $R–SO_3^-$) in solution. The SAX silica-based support has a quaternary amino-functionalized surface with Cl^- as a counter ion, which can be represented as silica–C_3H_6–$NR_3^+Cl^-$. As the sample passes through the SAX sorbent, the $R–SO_3^-$ displaces the Cl^- from the resin and therefore is likely removed from solution as follows:

$$\text{silica–}C_3H_6\text{–}NR_3^+Cl^- + R–SO_3^- \rightarrow \text{silica–}C_3H_6\text{–}NR_3^+\ ^-O_3S–R + Cl^-$$

Although carboxylic acids are weak acids, they are also completely dissociated at or near neutral pH, and interact with the SAX resin in the same fashion.

Weak black liquor

WBL contains higher levels and a broader range of organic and inorganic compounds than does spent sulphite liquor, and therefore represents the most difficult matrix one is ever likely to encounter. The effectiveness of PVP–SAX sorbents in purifying a hydrolyzed weak black liquor sample (neutralized to pH 6.5 with NaOH and diluted 1:125 with water) was assessed by measuring the UV–Vis absorption spectra before and after SPE, using the conditions described for Mg-SSL. The absorption spectra of the untreated, and PVP–SAX-treated WBL hydrolyzates are shown in Fig. 3B. The absorption spectrum of the untreated sample shows a number of strong absorption bands from 200–400 nm, primarily arising from the numerous aromatic compounds (such as lignin and phenols) present in the hydrolyzate. The spectra of the PVP–SAX-treated sample shows fewer and much less intense absorptions over the same wavelength range, indicating significant removal of aromatic compounds. In addition, the eluate from the sorbents was observed to be nearly colourless. The general scheme for purifying hydrolyzates is described in the Experimental section.

3.4. Effect of acid concentration on monosaccharide yield from process liquors

Suitable conditions for carrying out the hydrolysis of wood and various fibrous substances with sulphuric acid have been documented [1–4,12,20], and usually include primary and secondary hydrolysis steps. Hydrolysis of process liquors are generally carried out under the same secondary hydrolysis conditions (time and temperature) used for wood and pulp [12,20]. However, to our knowledge, the effect of sulphuric acid concentration on the yield of monosaccharides from the hydrolysis of process liquors and effluents has not been reported and was therefore examined for the three types of liquors used in this work, using secondary hydrolysis conditions only. This experiment was conducted on different samples from those referred to earlier in this paper. Aliquots of 10 ml of Mg-SSL and WBL were treated with 0.5, 1.0, 1.5 and 2.0 ml of 72% H_2SO_4, while 20-ml aliquots of PMWW were hydrolyzed with 0.25, 0.5, 1.0, 2.0 and 3.0 ml of 72% H_2SO_4. In each case the final volume was adjusted to 29 ml with water prior to hydrolysis at 121°C for 60 min. The monosaccharides in each hydrolyzate were quantified using SPE–HPAEC–PAD and the results for each sample were plotted against acid/sample (v/v) ratios. As indicated in Fig. 4, the concentration of each monosaccharide generally increases with increasing acid/sample ratio, reaches a maximum, and then decreases or levels off. In spite of the wide differences in chemistry (*e.g.*, alkalinity and dissolved solids content) and saccharide concentrations between the three samples, the optimum yield of monosaccharides appears to be reached at about the same acid/

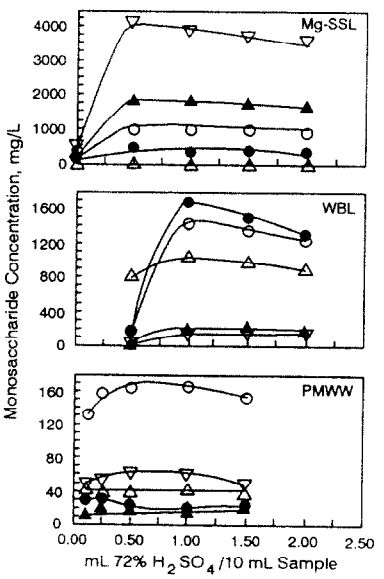

Fig. 4. Variation in the yield of wood monosaccharides from Mg-SSL, WBL and PMWW samples with varying amounts of 72% (w/w) H_2SO_4. All samples hydrolyzed for 60 min at 121°C. △ = Arabinose; ○ = galactose; ▲ = glucose; ● = xylose; ▽ = mannose. For HPAEC–PAD conditions, see Experimental section.

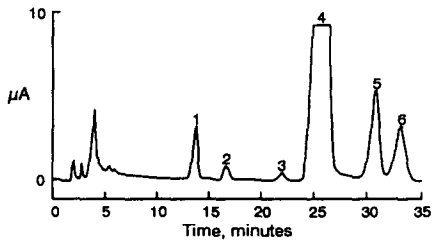

Fig. 5. SPE–HPAEC–PAD chromatogram of an unbleached kraft pulp hydrolyzate. Internal standard present at 5 mg/l. See Fig. 1 for peak identities and the Experimental section for analytical conditions.

sample ratio (1:10) for each sample. Accordingly, this ratio was used for the hydrolysis of the three types of liquors. Other liquors, particularly those with high solids content, may require higher ratios to achieve the same result.

3.5. Analysis of carbohydrates in wood, pulp and process liquors

Wood and pulp

A typical chromatogram of an unbleached kraft pulp hydrolyzate which serves to illustrate the selectivity of HPAEC is shown in Fig. 5. In spite of the high level of glucose present in the sample, the adjacent galactose peak is well resolved. Carbohydrate results obtained by HPAEC–PAD and GC methods for samples of aspen and pine wood, TMP (chlorite delignified) and unbleached kraft pulp are summarized in Table 4. As indicated in the Table, very good agreement between both methods was observed in all cases. The precision of both methods was determined by carrying out replicate analysis of the unbleached kraft pulp hydrolyzate. The relative standard deviation for the HPAEC–PAD and GC methods ranged between 3 to 7% and 2 to 12%, respectively.

Process liquors

Samples of unhydrolyzed and hydrolyzed Mg-SSL and WBL were subjected to PVP–SAX clean-up and then analyzed by HPAEC–PAD. Typical chromatograms of the hydrolyzed liquors are shown in Fig. 6A and B, respectively. Excellent resolution of all peaks was observed in both cases. For example, even though the Mg-SSL sample contained a relatively high level of xylose, no significant overlap with the adjacent mannose peak was observed. Unhydrolyzed Mg-SSL contained very low levels of each monosaccharide, while the unhydrolyzed WBL sample contained no monosaccharides, as expected.

The recovery of monosaccharides from Mg-SSL and WBL (pilot plant sample) was assessed by spiking dilute solutions of each hydrolyzed liquor with known amounts of each wood monosaccharide. Original and spiked hydrolyzates

Table 4
Comparison of HPAEC–PAD and GC carbohydrate results for aspen, pine, TMP and unbleached kraft pulp hydrolyzates

Monosaccharide	Monosaccharide concentration (%, w/w)							
	Aspen		Pine		TMP		Kraft	
	HPAEC ($n=2$)	GC ($n=2$)	HPAEC ($n=2$)	GC ($n=2$)	HPAEC ($n=4$)	GC ($n=2$)	HPAEC ($n=6$)	GC ($n=5$)
Arabinose	0.7	0.6	2.2	2.3	0.7	0.6	0.8	0.7
Galactose	1.1	ND[a]	2.7	2.3	0.4	0.4	0.6	0.6
Glucose	46.7	45.1	44.2	43.7	84.9	87.4	73.8	73.3
Xylose	14.7	15.8	6.0	6.9	8.7	8.0	7.3	7.4
Mannose	2.8	2.5	12.2	11.4	6.8	6.1	6.0	5.8
Total	66.0	64.0	67.3	66.6	101.5	102.5	88.5	87.8

[a] ND = Not detected.

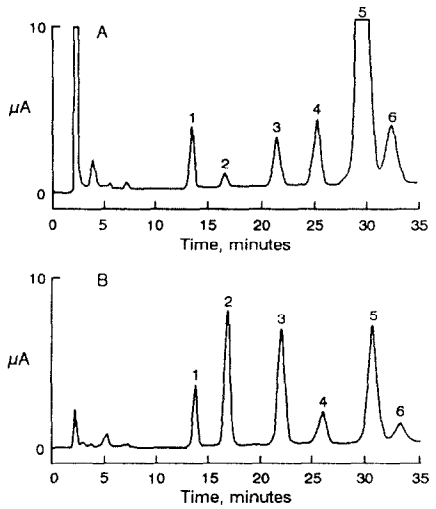

Fig. 6. SPE–HPAEC–PAD chromatograms of hydrolyzed Mg-SSL (A) and WBL (B). Internal standard present at 5 mg/l in both samples. See Fig. 1 for peak identities and the Experimental section for analytical conditions.

were then subjected to SPE using PVP–SAX, and analyzed for carbohydrate content. All of the wood monosaccharides were quantitatively recovered from both types of hydrolyzates, indicating the effectiveness of the SPE–HPAEC–PAD method. The carbohydrate content of hydrolyzed Mg-SSL, WBL and PMWW was also determined using the GC method. The results, presented in Table 5, show that the two methods are in good agreement. The precision of the combined hydrolysis and SPE–HPAEC–PAD method was assessed by carrying out replicate ($n = 4$) hydrolysis and subsequent carbohydrate analysis, using a sample of mill WBL. The results, presented in Table 6, show that the overall precision ranged between 2 and 7%, and the total monosaccharide result had an R.S.D. of about 3%. The precision results for mannose were not determined, since it was present below the detectable level in this particular sample.

In the case of the PMWW hydrolyzate, monosaccharides originate mainly from dissolved hemicellulose components. The unhydrolyzed PMWW sample was also analyzed, and found to contain no monosaccharides. The ability to routinely use HPAEC–PAD to determine carbohydrates in highly contaminated process liquors is due primarily to the effectiveness of the sample clean-up technique described in this work. After performing several hundred analyses of wood, pulp and process liquor samples using the same column, we have not observed any significant decrease in efficiency, and continue to use the same guard and analytical columns.

4. Conclusions

We have demonstrated that wood monosaccharides can be baseline resolved under isocratic

Table 5
Comparison of carbohydrate results for hydrolyzed Mg-SSL, WBL (pilot plant sample), PMWW, obtained using SPE–HPAEC–PAD and GC methods

Monosaccharide	Monosaccharide concentration (mg/l)					
	Mg-SSL		WBL		PMWW	
	HPAEC	GC	HPAEC	GC	HPAEC	GC
Arabinose	333	426	1005	1095	110	102
Galactose	1054	1053	1366	1294	165	141
Glucose	2023	1908	146	140	32	39
Xylose	772	782	1685	1773	18	15
Mannose	4524	4523	105	80	36	47
Total	8706	8692	4307	4382	361	344

Table 6
Overall precision obtained for replicate analysis of WBL (mill sample) hydrolyzate, using the SPE–HPAEC–PAD method

Monosaccharide	Monosaccharide concentration (mg/l)					
	Analysis No.				Mean	R.S.D. (%)
	1	2	3	4		
Arabinose	1177	1148	1161	1213	1175	2.4
Galactose	711	777	794	804	772	5.4
Glucose	40	35	40	37	38	6.5
Xylose	1410	1497	1519	1563	1497	4.3
Total	3338	3457	3514	3617	3482	3.3

All hydrolyses and analyses were performed on the same day.

conditions in less than 40 min. Very reproducible retention times, peak areas and response factors were obtained by using a post-run column flush with 350 m*M* NaOH. The PAD response was linear for each monosaccharide over the concentration range of 0.5 to 150 mg/l. The applicability of the HPAEC–PAD technique has been expanded to include the analysis of carbohydrates in process liquors through the development of a simple, inexpensive SPE technique which utilizes PVP–SAX sorbents. Carbohydrates were quantitatively recovered from pulp and process liquor hydrolyzates using the SPE technique. Carbohydrate results obtained for wood, pulp, Mg-SSL, WBL and PMWW, using HPAEC–PAD and GC methods were in excellent agreement. The overall precision of the SPE–HPAEC–PAD method for pulp and weak black liquor ranged between 2 to 7% expressed as R.S.D. of individual monosaccharide concentrations. Optimum monosaccharide yield from the three types of process liquors was demonstrated to occur at the same acid/sample ratio (1 ml 72% H_2SO_4 per 10 ml sample).

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6. References

[1] J.F. Saeman, W.E. Moore, R.L. Mitchell and M.A. Millett, *Tappi J.*, 37 (1954) 336.
[2] L.G. Borchardt and C.V. Piper, *Tappi J.*, 53 (1970) 257.
[3] C. Hoebler, J.L. Barry, A. David and J. Delort-Laval, *J. Agric. Food Chem.*, 37 (1989) 360.
[4] P.J. Harris, A.B. Blakeney, R.J. Henry and B.A. Stone, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 272.
[5] J.T. Sweeley, W.E. Moore, R.L. Mitchell and M.A. Millett, *J. Am. Chem. Soc.*, 85 (1963) 2497.
[6] G.L. Cowie and J.I. Hedges, *Anal. Chem.*, 56 (1984) 497.
[7] J.M. MacLeod and J.V. Benko, *J. Wood Chem. Technol.*, 2 (1982) 207.
[8] R.B. Kesler, *Anal. Chem.*, 39 (1967) 1416.
[9] P.L. Van Biljon and S.P. Olivier, *J. Chromatogr.*, 473 (1989) 305.
[10] L.I. Larsson and O. Samuelson, *Svensk Papperstidn.*, 70 (1967) 571.
[11] M. Sinner, M.H. Simatupang and H.H. Dietrichs, *Wood Sci. Technol.*, 9 (1975) 307.
[12] B. Krogerus, *Paperi ja Puu*, 66 (1984) 649.
[13] G. Marko-Varga, E. Dominguez, B. Hahn-Hägerdal and L. Gorton, *Chromatographia*, 30 (1990) 591.
[14] G. Bonn, R. Pecina, E. Burtscher and O. Bobleter, *J. Chromatogr.*, 287 (1984) 215.
[15] D.W. Patrick and W.R. Kracht, *J. Chromatogr.*, 318 (1985) 269.
[16] G. Marko-Varga, E. Dominguez, B. Hahn-Hägerdal, L. Gorton, H. Irth, G.J. de Jong, R.W. Frei and U.A.Th. Brinkman, *J. Chromatogr.*, 523 (1990) 173.
[17] W.E. Karr, L.G. Cool, M.M. Merriman and D.L. Brink, *J. Wood Chem. Tech.*, 11 (1991) 447.
[18] T. Lindén and B. Hahn-Hägerdal, *Biotechnol. Tech.*, 3 (1989) 189.
[19] H. Binder, *J. Chromatogr.*, 189 (1980) 414.

[20] F.E. Wentz, A.D. Marcy and M.J. Gray, *J. Chromatogr. Sci.*, 20 (1982) 349.
[21] R.C. Pettersen, V.H. Schwandt and M.J. Effland, *J. Chromatogr. Sci.*, 22 (1984) 478.
[22] M.G. Paice, L. Jurasek and M. Desrochers, *Tappi J.*, 65 (1982) 103.
[23] D.C. Johnson and W.R. LaCourse, *Anal. Chem.*, 62 (1990) 589A.
[24] J.D. Olechno, S.R. Carter, W.T. Edwards, D.G. Gillen, R.R. Townsend, Y.C. Lee and M.R. Hardy, in T.E. Hugli (Editor), *Techniques in Protein Chemistry*, Academic Press, San Diego, CA, 1989, p. 367.
[25] D.A. Martens and W.T. Frankenberger, *Chromatographia*, 29 (1990) 7.
[26] R.D. Rocklin and C.A. Pohl, *J. Liq. Chromatogr.*, 6 (1983) 1577.
[27] W.T. Edwards, C.A. Pohl and R. Rubin, *Tappi J.*, 70 (1987) 138.
[28] R.C. Pettersen and V.H. Schwandt, *J. Wood Chem. and Tech.*, 11 (1991) 495.
[29] M. Ishihara, S. Uemura, N. Hayashi and K. Shimizu, *Biotechnol. Bioeng.*, 37 (1991) 948.
[30] D.A. Martens and W.T. Frankenberger, *Talanta*, 38 (1991) 245.
[31] D.A. Martens and W.T. Frankenberger, *J. Chromatogr.*, 546 (1991) 297.
[32] K. Koizumi, Y. Kubota, T. Tanimoto and Y. Okada, *J. Chromatogr.*, 464 (1989) 365.
[33] K. Koizumi, M. Fukuda and S. Hizukuri, *J. Chromatogr.*, 585 (1991) 233.
[34] R.J. Wicks, M.A. Moran, L.J. Pittman and R.E. Hodson, *Appl. Environ. Microbiol.*, 57 (1991) 3135.
[35] G.O.H. Peelen, J.G.N. deJong and R.A. Wevers, *Anal. Biochem.*, 198 (1991) 334.
[36] M.R. Hardy, R.R. Townsend and Y.C. Lee, *Anal. Biochem.*, 170 (1988) 54.
[37] G.P. Reddy and C.A. Bush, *Anal. Biochem.*, 198 (1991) 278.
[38] K.W. Swallow, N.H. Low and D.R. Petrus, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 341.
[39] D.R. White and W.W. Widmer, *J. Agric. Food Chem.*, 38 (1990) 1918.
[40] R.M. Pollman, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 425.
[41] K.W. Swallow and N.H. Low, *J. Agric. Food Chem.*, 38 (1990) 1828.
[42] S. Mou, Q. Sun and D. Lu, *J. Chromatogr.*, 546 (1991) 289.
[43] K.A. Garleb, L.D. Bourquin and G.C. Fahey, *J. Agric. Food Chem.*, 37 (1989) 1287.
[44] S.C. Churms, *J. Chromatogr.*, 500 (1990) 555.
[45] Y.C. Lee, *Anal. Biochem.*, 189 (1990) 151.
[46] D.C. Johnson, D. Dobberpuhl, R. Roberts and P. Vandeberg, *J. Chromatogr.*, 640 (1993) 79.
[47] W. Bichard, *B.Sc. Thesis*, Concordia University, Montreal, April 1990.
[48] J.M. Hachey, V.T. Bui, Y. Tremblay, D. Houde and W.G. Mihelich, *J. Wood Chem. Technol.*, 6 (1986) 389.
[49] G.W. Sanderson and B.P.M. Perera, *Analyst*, 91 (1960) 335.
[50] C. Ciavatta, M. Govi, L.V. Antisari and P. Sequi, *J. Chromatogr.*, 509 (1990) 141.
[51] M.N. Clifford, *J. Chromatogr.*, 94 (1974) 261.