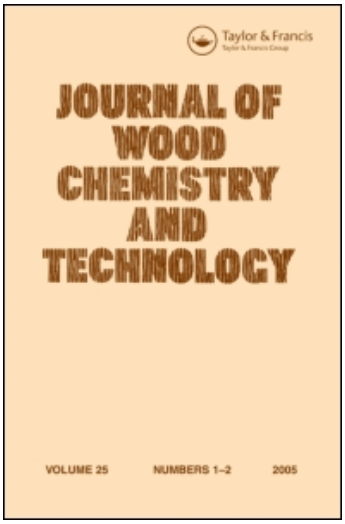

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WOOD SUGAR ANALYSIS BY ANION CHROMATOGRAPHY

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ABSTRACT

Two wood and two wood-pulp samples were hydrolyzed in triplicate in sulfuric acid. The sugar monomers were separated by anion-exchange chromatography and quantitatively measured with a pulsed amperometric detector. A portion of each sugar solution was neutralized with sodium hydroxide and analyzed for the sugar content in the same manner as the unneutralized hydrolysates. The results from analyses of acid and neutral solutions were consistent. Detection limits for the individual sugars ranged from 25 ng for arabinose to 13 ng for glucose using a signal to noise ratio of three for the detection limit.

INTRODUCTION

Wood scientists often require a detailed quantitative analysis of the various monosaccharide units in woods, wood pulps, and related biomass materials. Such information is useful to monitor the conditions used in a biopulping or fermentation process, to measure the effects of treatments of wood with chemical preservatives or fire-retardants, or to define the kinetic equations for processes that make use of wood or lignocellulosic material as chemical feedstocks. This information is also useful for the study of wood decay by various fungal or bacterial strains.

The Forest Products Laboratory has performed numerous chemical analyses of the polysaccharide content of woods and pulps. Briefly, the procedure is to

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hydrolyze the sample in acid solution, isolate the sugar monomers, and separate and quantitate the individual sugars. The original procedure used paper chromatography to separate the sugars and a photometric detection of the sugar-reducing power to quantitate them.[1] Twelve years ago, we began to investigate the use of HPLC to separate and detect the sugar monomers. Soon thereafter, we converted our method to separation and quantitation by cation-exchange liquid chromatography and refractive index detection.[2] This change greatly improved our efficiency because the automated equipment eliminates the time-consuming elution of individual sugars from paper tabs. Recently, we further improved the efficiency of sugar separation and detection with anion-exchange liquid chromatography and pulsed amperometric detection (PAD).

This report concerns the results we obtained from standard wood and wood-pulp samples using anion-exchange liquid chromatography and PAD. The basis for use of the anion column and PAD was outlined in a paper in 1983.[3] A recent review summarized advances for the broader aspects of anion-exchange chromatography of carbohydrates.[4]

EXPERIMENTAL

Wood samples of yellow birch (*Betula alleghaniensis*, Britton) and loblolly pine (*Pinus taeda*, L.), a neutral sulfite semichemical (NSSC) pulp of southern red oak (*Quercus falcata*, Michx.), and a kraft pulp of loblolly pine were analyzed for five wood sugars—glucose, xylose, galactose, arabinose, and mannose. All the samples had been ground in a Wiley mill** to pass a 40-mesh screen. The yellow birch was extracted with a 2:1 benzene/ethanol solvent, and the loblolly pine was extracted with 9:1 acetone/water and then 1:1 toluene/ethanol. All samples were vacuum dried at 50°C for 4 h before weighing. Approximately 50 mg of each was hydrolyzed with 0.50 mL of 72% H_2SO_4 for 1 h in a water bath held at 30°C. The samples were then diluted to 4% H_2SO_4 with distilled water and placed in an autoclave at 120°C for 1 h. Samples were run in triplicate, all at the same time. The insoluble lignin was filtered off and washed with hot distilled water, and the wash was combined with the filtrate. Each hydrolysate was divided into two portions after adding a fucose internal standard. Measured volumes (25 μL)

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from one portion were injected directly onto a Dionex HPIC–AS–6 anion column (Dionex Corp., Sunnyvale, CA). The other portion was neutralized with sodium hydroxide before injection onto the same column. (Note: Wood or pulp samples treated with special chemicals such as preservatives or enzymes may require filtering of the acid hydrolysate through a Waters C–18 Sep–pak, Millipore Corp., Milford, MA, or equivalent before injection onto the anion column.) A standard solution containing the five sugars in the approximate proportions found in wood, plus the fucose internal standard, was used to determine response factors. The standard was injected after every 10 unknown sample injections to monitor possible variations in the column or detector conditions.

The chromatographic equipment consisted of a model 4000i Dionex pump and controller system, a Dionex model UEM–1 PAD, and a Waters model 710 WISP auto sample injector. The column was from Dionex, an HPIC–AS6 (250 by 4 mm i.d.), packed with 10-μm substrate spheres coated with anion latex. The analytical column was protected by an AG6 guard column (50 by 4 mm i.d.). The PAD had a flow-through cell equipped with a gold working electrode, a stainless steel counter electrode, and a silver/silver chloride reference electrode. The working electrode potential was cycled through three values to allow cleaning of the electrode surface and current stabilization before sampling the oxidative current from the flow-through solution. Working electrode potentials were set as follows: $E_1 = 0.20$ V (300 ms), $E_2 = 0.60$ V (120 ms), and $E_3 = -0.9$ V (300 ms) with a sampling time of 16.7 ms (1/60 s to eliminate 60-cycle line voltage variation) at the end of E_1. The E_2 and E_3 pulses remove remaining sugars and then reactivate the gold surface, respectively. We carefully optimized their values for maximum sensitivity.

Although an earlier report[3] indicated the use of weak sodium hydroxide (0.25 mM) as an eluent to improve selectivity and the addition of sodium acetate to control retention time, we have found that water as an eluent gave adequate resolution and acceptable retention times. However, the column required regeneration with 0.25 M NaOH after each run. Apparently enough carbonate is introduced with each injection such that good sugar separation is not possible on a subsequent injection. The eluent, therefore, must be entirely free from carbon dioxide. Freshly filtered-deionized water was placed in a plastic reservoir and degassed with helium for 15 min before use. After being prepared in this manner, the eluent in the reservoir was kept sealed from outside air. The eluent flow rate was 1.0 mL/min and 0.3 M NaOH was introduced into the postcolumn stream at a flow rate of

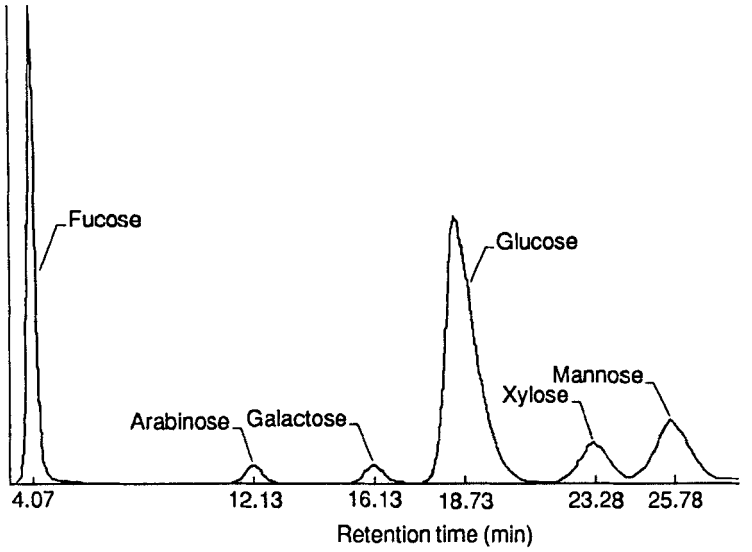

Figure 1. Anion chromatographic trace of sugars in loblolly pine wood. Experimental conditions are described in the text.

0.5 mL/min to enhance sugar detection in the PAD cell. The total time between injections was about 75 min, which included 28 min of sample run after injection, 7 min of column regeneration, and 40 min of baseline stabilization with water eluent.

A chromatographic trace of loblolly pine wood is shown in Figure 1, with retention times in minutes given for the fucuse, arabinose, galactose, glucose, xylose, and mannose peaks. Our original column continues to perform as new after more than 4,000 injections.

The chromatographic system was controlled by a Perkin Elmer/Nelson chromatography workstation (Cupertino, CA) and associated Epson II computer and printer–plotter. All detector data were collected and stored on diskettes for later retrieval and interpretation. Generally, chromatographic peaks were baseline corrected and integrated for area. Integrated peak areas from standard sugar solutions were used to compute the response factors.

TABLE 1
Polysaccharide Content of Woods and Wood Pulps as Measured by Anion Exchange Chromatography

Sample[b]	Condition	Polysaccharide content (%)[a]				
		Glucan	Xylan	Galactan	Arabinan	Mannan
Pine	Acid	41.6(9)	7.0(2)	2.2(1)	1.7(1)	10.6(2)
	Neutral	41.0(7)	6.3(3)	2.2(1)	1.7(1)	9.6(3)
	(Previous)[c]	40.7(6)	6.9(1)	2.4(6)	1.3(1)	11.0(5)
Birch	Acid	43.8(8)	21.1(2)	1.1(0)	0.7(0)	1.0(1)
	Neutral	40.9(7)	20.2(5)	1.1(1)	0.7(1)	0.8(2)
Pine kraft pulp	Acid	64.3(6)	8.1(1)	1.0(1)	0.8(0)	6.6(2)
	Neutral	62.0(3)	7.8(2)	1.0(2)	0.8(2)	6.1(3)
	(Previous)[c]	62.0(6)	7.2(2)	0.9(1)	0.7(1)	6.2(1)
Oak NSSC pulp	Acid	52.5(6)	23.9(2)	0.6(0)	0.5(0)	0.4(1)
	Neutral	50.1(4)	22.9(0)	0.5(0)	0.5(0)	0.5(1)

[a]Percentage of anhydro sugar on ovendry basis. Standard deviations are in parentheses and apply to the last significant figure.

[b]Samples were loblolly pine, yellow birch, loblolly pine kraft pulp, and southern red oak neutral sulfite semichemical (NSSC) pulp.

[c]Values taken from a previously published method using cation exchange chromatography and refractive index detection.[1]

RESULTS

The polymeric carbohydrate contents of the four samples are tabulated in Table 1. Values are reported from measurements on acid hydrolysates and neutral hydrolysates. Each hydrolysate was injected three times, and each sample was done in triplicate. These multiple determinations were used to compute the standard deviation of the measurement, which is given in parentheses. Two samples, loblolly pine wood and kraft pulp, had been previously analyzed by cation chromatography using a Pb(II)-impregnated column. Those results are also included in Table 1. Inspection of the results shows that it is unnecessary to neutralize the hydrolysate before injection onto the anion column. The anion results are also in very good agreement with the previous results from cation chromatography for the two pine samples.

We investigated the detection limit for each sugar and the fucose internal standard by injecting a dilute standard solution and measuring the ratio of signal

TABLE 2
Lower Limit of Detection for Each Sugar Based on a Signal/Noise Ratio of 3

		Measurements at different PAD settings					
		10,000-nA full-scale			300-nA full-scale		
Sugar	Injected solution (ng)	Peak height	Baseline noise	Detection limit (ng)	Peak height	Baseline noise	Detection limit (ng)
Fucose	42.6	135	8	5.4	6,514	141	2.8
Arabinose	15.4	24	17	25.3	1,068	141	7.0
Galactose	24.2	29	9	14.2	1,682	141	6.6
Glucose	102.0	106	7	13.1	5,816	141	7.6
Xylose	24.8	24	8	16.5	1,193	141	10.0
Mannose	28.5	29	10	23.1	999	141	14.0

to noise. The noise was the largest peak to valley span observed in the detector baseline trace, and the signal was the peak height above baseline for each analyte. The lower limit of detection was taken to be a conservative three times the noise level. Table 2 summarizes the results of these measurements and the detection limits for two sensitivity settings of the detector. The full-scale setting of 10,000 nA is more representative of a real analysis situation and is the setting commonly used for routine analyses. The 300-nA setting improves the lower detection limit by about a factor of two, but it is not practical for day-to-day operation because of fluctuating baselines when real samples are injected.

CONCLUDING REMARKS

We demonstrated the precision and sensitivity possible for the quantitative analysis of the monosaccharide components of wood and wood-pulp samples. Fifty milligrams of sample were hydrolyzed with sulfuric acid, and the sugar monomers were separated by anion-exchange chromatography and detected with a pulsed amperometric detector. It was not necessary to neutralize or concentrate the hydrolysate before column injection. Relative standard deviations ranged from about 22% for minor components, which were <1% of the sample dry weight, to 0.5% for the major component (glucan), which was >50% of the sample dry weight. Detection limits are of the order of 10 ng for each sugar.

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