Determination of carbohydrates in wood pulp products

Murray L. Laver and Kenneth P. Wilson

ABSTRACT: The authors measured carbohydrates in rayons and a dissolving pulp using acid hydrolysis, anion exchange chromatographic separation of the resulting monosaccharides, and pulsed amperometric detection of the separated monosaccharides. Analysis of the hydrolyzates without acid neutralization or monosaccharide derivatization proved effective in separating and analyzing low concentrations of D–xylose and D–mannose in the presence of D–glucose. This allowed close approximations of hemicellulose in cellulosic products. The rayons contained less than 4.0% hemicellulose and the dissolving pulp less than 3.5% hemicellulose.

KEYWORDS: Acidolysis, amperometry, anion exchange, carbohydrates, chromatography, detection, dissolving pulps, hemicelluloses, monosaccharides, quantitative analysis, rayon, wood.

Determination of carbohydrates in wood pulp products provides information for processing, uses, and derivatization. The amounts of cellulose and hemicellulose are particularly important. The usual way to measure these is by acid hydrolysis followed by identification and quantification of the resulting monosaccharides. Such results are not transformable into exact quantities of cellulose and hemicellulose because several hemicelluloses, as well as cellulose, release D–glucose. Knowledge about the chemistry of wood hemicelluloses (*1, 2*), however, can lead to useful information about cellulose and hemicellulose contents.

Several articles have reviewed the previous methods for acid hydrolysis of wood pulp products and subsequent analysis of the resulting monosaccharides (*3–5*). The procedures have usually included: dissolution and primary hydrolysis in strong acid, complete hydrolysis by heating in dilute acid, neutralization of the acid, evaporation of the neutralized solution, derivatization of the monosaccharides, separation and measurement of the monosaccharide derivatives, and calibration and calculation. These steps are in a standard test method (*6*) based on gas chromatography of alditol acetates. The test involves acid hydrolysis, neutralization of the hydrolyzate, and the reduction of the monosaccharides to alditols with sodium borohydride. One must then completely evaporate the aqueous solvent. There is subsequent acetylation of the alditols, extraction into an organic solvent, and injection into a gas chromatograph. This analytical procedure is time consuming and involves numerous steps. Each step requires skilled care if the procedure is to be quantitative.

The advent of anion exchange chromatography for the separation of carbohydrates and the commercial availability of the pulsed amperometric detector (PAD) have changed the analysis of carbohydrates. In recent publications, one researcher (*7*) reviews high–performance anion exchange chromatography for carbohydrates, and a research team (*8*) specifically describes wood sugar analysis.

Our work concerns results we obtained from the analyses of several commercial rayon samples and a commercial dissolving pulp in which the amount of D–glucose in each hydrolyzate was many times larger than the amount of monosaccharides released from the hemicelluloses. This imbalance has been a continuing problem affecting analysis of polysaccharide materials such as dissolving pulps and regenerated cellulosics that are almost exclusively of one monosaccharide repeating unit, usually D–glucose. Previous methods (*3, 4, 6*) failed to separate adequately, and thereby quantify, trace quantities of D–xylose, D–mannose, D–galactose, and L–arabinose resulting from the hydrolysis

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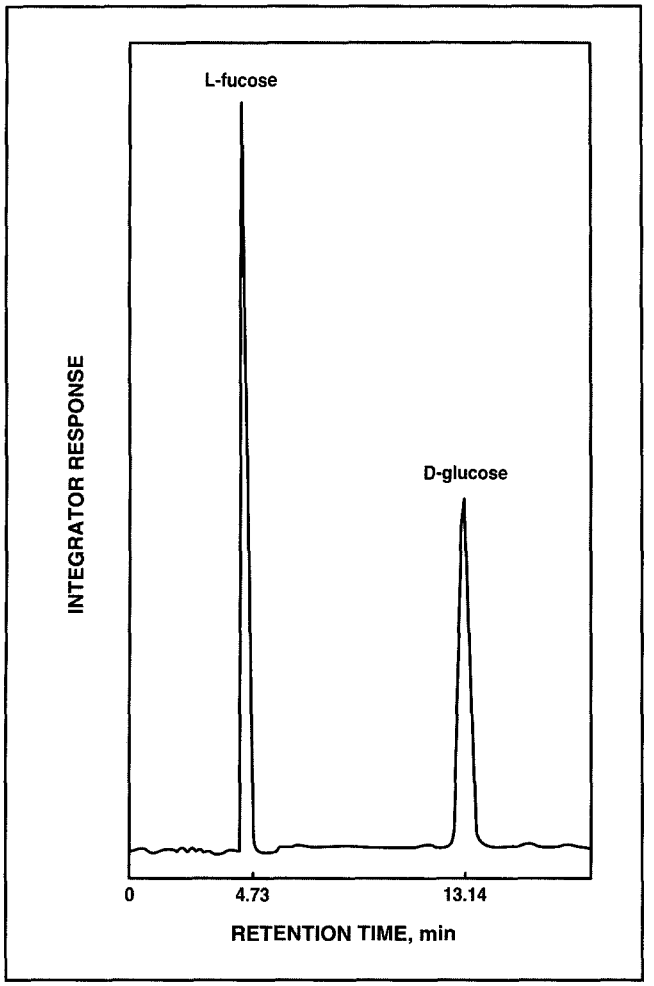
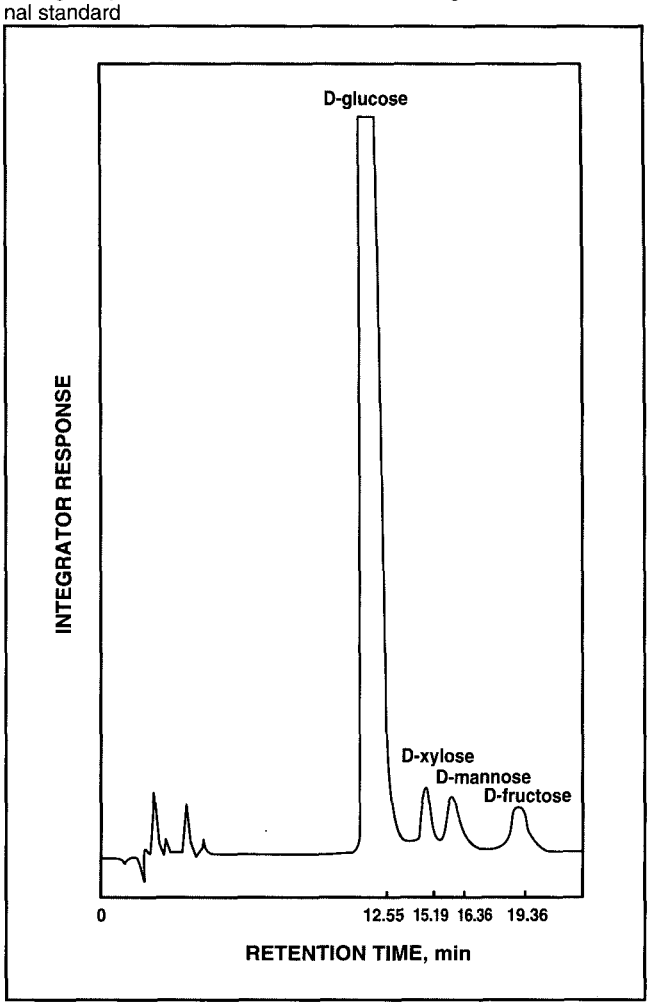
1. Anion exchange chromatogram of a rayon hydrolyzate for determining D–glucose content using L–fucose as internal standard



2. Anion exchange chromatogram of a rayon hydrolyzate for determining D–xylose and D–mannose contents using D-fructose as internal standard



of the small quantities of hemicelluloses in dissolving pulps and rayon materials. This report provides a sequence of procedures that includes sample preparation, careful hydrolysis, separation of monosaccharides, detection, calibration, and calculation. This sequence accomplishes the analyses of the monosaccharides from the hemicelluloses in the presence of much greater quantities of D–glucose.

Results and discussion

This study evaluated the use of anion exchange chromatography coupled with PAD to analyze quantitatively the trace amounts of monosaccharides released from hemicellulose hydrolysis in the presence of as much as 95 times the amount of D–glucose released from cellulose hydrolysis. This is an overwhelming amount of D–glucose. These results allowed the calculation of the percentage of cellulose and hemicellulose in the rayon and dissolving pulp starting materials.

Sample preparation and hydrolysis

The study materials included 10 commercial rayon samples and a commercial dissolving pulp from a firm interested in rayon utilization. We included the dissolving pulp, a representative of the starting material for making rayon samples, for comparison of its hemicellulose content with the hemicellulose contents of the rayons after processing.

Soxhlet extraction with 1,1,1-trichloroethane and 2–propanol removed the surface coatings from processing. Dissolution in concentrated sulfuric acid (77%) accomplished the hydrolysis with subsequent dilution to 3.0% and refluxing for five hours (*3*). If dilution resulted in white, flocculent solids (cellulose particles), we discarded the sample and replaced it with one exposed for three hours to the concentrated acid. This ensured that the cellulose polymers were short enough to remain soluble. After reflux, we diluted the hydrolyzates to a standard volume of 250.0 mL and took aliquots for the addition of an appropriate internal standard (L–fucose or D–fructose) and further dilution (1.0–15.0 μg/mL of monosaccharide concentration) before analysis by liquid chromatography.

Liquid chromatography

The chromatographic equipment was similar to that described elsewhere (*8, 9*). A previous technical note (*10*) described the eluants (dilute aqueous sodium hydroxide containing sodium acetate) and post–column solution (0.3 *M* sodium hydroxide). The timed program of elution, column regeneration, equilibration, and repeated cycles is new to this work.

Other investigators (*8*) recently reported that water as eluant separated wood sugar monosaccharides. The water eluant required column regeneration after each injection. The total injection recycle time was 75 min. The timed program in this work allowed a new injection in 60 min or less. When only D-glucose was analyzed, the total concentration of sugars was low (1.0–15.0 μg/mL). Regeneration was then necessary only after six or more injections. The recycle time was then 20 min.

Instrument calibration equations

Addition of internal standards (L–fucose or D–fructose) reduced apparatus and procedural errors. Early experiments showed that the hydrolyzates contained D–glucose, D–xylose, and D–mannose but no D–galactose or L–arabinose, although all five might be present in wood pulp products (*3, 4, 8*). D–glucose was present in overwhelming quantities. Two separate hydrolyzate dilutions were necessary. Concentrations of hydrolyzate that allowed measurement of D–xylose and D–mannose resulted in a D–glucose response that exceeded the maximum calibration limits of the integrator. Concentrations that kept D–glucose quantities within limits did not contain enough D–xylose and D–mannose for measurement. Thus, two separate injections were necessary. One was for the analysis of D–glucose, and the other was for the analysis of D–xylose and D–mannose. All efforts to achieve a hydrolyzate dilution that allowed measurement of D–glucose, D–xylose, and D–mannose in a single analytical injection failed because of the overwhelming amount of D–glucose. The time for the additional injections did not prove prohibitive, however, because of the short time (20 min) for each D–glucose analysis. L–fucose was the internal standard for D–glucose analysis as shown in **Fig. 1**, and D–fructose was the standard for the D–xylose and D–mannose analyses as shown in **Fig. 2**.

Calculations with the method of least squares using peak areas from the analyses of five standard solutions containing authentic D–glucose and L–fucose in increasing weight ratios of 0.63 to 1.89 resulted in the following straight–line equation for D–glucose:

$$Y = -0.075 + X \qquad (1)$$

where

Y = peak area of the test monosaccharide/peak area of the internal standard

X = weight of the test monosaccharide/weight of the internal standard

Similarly, calculations using the peak areas from the analyses of five standard solutions containing authentic D–xylose, D–mannose, and D–fructose in increasing weight ratios of 0.34 to 1.03 D–xylose/D–fructose, and 0.33 to 0.98 D–mannose/D–fructose resulted in the straight–line relationship in Eq. 2 for D–xylose and the relationship in Eq. 3 for D–mannose.

$$Y = 0.165 + 1.510X \qquad (2)$$

$$Y = 0.050 + 1.382X \qquad (3)$$

The R values for Eqs. 1, 2, and 3 are 0.999, 0.999, and 0.997, respectively.

Polysaccharide percentage

To understand better the composition of the samples before their hydrolyses, we converted the monosaccharides in the hydrolyzates to percent polysaccharides: D–glucose to glucan, D–xylose to xylan, and D–mannose to mannan. We converted monosaccharide peak areas from anion exchange chromatograms similar to those of Figs. 1 and 2 to monosaccharide weights using standard equations ascertained with the appropriate internal standards (L–fucose for D–glucose and D–fructose for D–xylose and D–mannose). We then converted the monosaccharide weights to polysaccharide percentage by considering hydrolyzate sample dilution, water of hydrolysis factors, and the original sample dry weight. The dilution was 1000 to 1 for D–glucose and 10 to 1 for both D–xylose and D–mannose. Water of hydrolysis factors were 0.90 for hexoses and 0.88 for pentoses.

Table I shows the polysaccharides as glucan, xylan, and mannan, although hemicelluloses may contain all three types of monosaccharide units. There is no way of knowing from the hydrolyzates which monosaccharides are chemically linked as hemicelluloses. All the D–xylose and D–mannose, however, came from hemicelluloses. The low quantities of xylan and mannan in the table show that the total amount of hemicelluloses was not large. The D–mannose in the hydrolyzates undoubtedly came from a glucomannan hemicellulose, so some of the D–glucose was associated with hemicelluloses. Another investigator (*1, 2*) reviewed glucomannan hemicelluloses and concluded that the ratios of D–glucose units were usually 1:2 in hardwoods and 1:3 in softwoods. These ratios, the data for rayon sample no. 8 in the table, and the corresponding data for the dissolving pulp in the table allowed calculation of the hemicellulose contents of less than 4.0% for the rayon samples and less than 3.5% for the dissolving pulp. The materials were therefore composed overwhelmingly of cellulose.

The total polysaccharides ranged from 95.66% to 97.43% of the starting material as seen in the table. This exceptional recovery rate accounted for almost all the starting materials. There was evidently a minimum of carbohydrate degradation during hydrolysis and little loss of monosaccharides during sample preparation and analyses. Analysis results from multiple injections (minimum of three injections)

ranged from +0.07% to +1.36% as seen in the table. This is excellent reproducibility by the chromatograph for separations and analyses as complex as these. The method is therefore a simple, quantitative technique for the determination of carbohydrates in wood pulp products.

Conclusions

The appendix to this article shows the experimental procedure of this work. Based on the results of the work we are able to make certain conclusions.

Anion exchange separation and PAD provided an analytical procedure for determining carbohydrates in wood pulp products with a minimum of sample preparation, improved accuracy, and a method to calculate hemicellulose content. It was necessary to hydrolyze the samples with acid, but the hydrolyzates diluted easily with water. The acid did not require neutralization, and the monosaccharides did not need derivatization. Analyzing the monosaccharides in their reducing sugar forms not only saved time but also improved accuracy because there was no need for a quantitative derivatization reaction. The results showed that an essentially routine procedure can allow analysis of large numbers of samples relatively quickly. The method also provided measurements of small quantities of D–xylose and D–mannose in the presence of large quantities of D–glucose, which allowed for the calculation of hemicellulose contents. TJ

I. Polysaccharide content of wood pulp products

Rayon sample	*% Glucan*	*% Xylan*	*% Mannan* *	*% Total recovery*
1	94.20 ± 0.55	1.29 ± 0.23	1.04 ± 0.20	96.53
2	94.66 ± 0.59	1.44 ± 0.46	1.05 ± 0.14	97.15
3	94.82 ± 1.36	1.37 ± 0.16	0.97 ± 0.13	97.16
4	94.30 ± 0.29	1.01 ± 0.46	0.67 ± 0.33	95.98
5	94.85 ± 1.08	0.96 ± 0.35	0.58 ± 0.14	96.39
6	93.64 ± 0.30	1.17 ± 0.32	0.95 ± 0.20	95.76
7	93.78 ± 0.27	1.51 ± 0.50	1.24 ± 0.29	96.53
8	93.02 ± 0.74	1.89 ± 0.56	1.30 ± 0.28	96.21
9	95.85 ± 1.14	0.90 ± 0.12	0.68 ± 0.07	97.43
10	95.76 ± 1.28	0.68 ± 0.11	0.58 ± 0.10	97.02
Dissolving pulp	92.66 ± 0.38	1.73 ± 0.56	1.27 ± 0.55	95.66

*A mannose–containing hemicellulose, undoubtedly a glucomannan, calculated as a mannan.

II. Conditions of analysis

PAD settings

E1 = +0.20 V	t1 = 300 ms
E2 = +0.60 V	t2 = 300 ms
E3 = -0.80 V	t3 = 480 ms

Eluant solutions

Solution 1:	Distilled, deionized 18-MQ quality water
Solution 2:	0.05*M* aqueous sodium hydroxide (low carbonate), to which was added acetic acid to a concentration of 0.015*M* (resulting in sodium acetate in the solution)
Solution 3:	0.1*M* aqueous sodium hydroxide (low carbonate)

Sequence

Minutes	Event
0	Monosaccharide solution injection
0–30	93% Solution 1 and 7% solution 2 (monosaccharides elution)
30–33	Eluant change to 100% solution 3
33–45	100% Solution 3 for column regeneration
45–48	Eluant return to 93% solution 1 and 7% solution 2
48–60	93% Solution 1 and 7% solution 2 for system equilibration
60	Clock reset to 0 for next injection

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Appendix

Sample preparation and hydrolysis

The procedure involved cutting approximately 4.0-g aliquots of all samples shown in Table I into pieces about 3 cm long and consecutively extracting in a Soxhlet apparatus with 1,1,1–trichloroethane followed by 2–propanol (30 solvent exchanges each), air–drying, and then oven–drying at 110°C for 24 hours. Then we kneaded approximately 200 mg of each dried sample into 77.0% sulfuric acid (5.0 mL) in 250-mL round–bottomed flasks submerged in ice–water baths. The flasks came to room temperature to enhance complete dissolution. After the samples had completely dissolved (about two hours), we resubmerged the flasks in the ice–water baths and added 123.0 mL water to each flask and stirred. After refluxing the solutions (3.0% in sulfuric acid) for five hours, we diluted the resulting hydrolyzates to 250.0 mL in volumetric flasks.

Then we diluted 10-mL aliquots of each hydrolyzate to 100.0 mL and added 10.0-mL aliquots of these diluted solutions to 100.0-mL volumetric flasks along with 10-mL aliquots of a standard solution of L–fucose (78.38 μg/mL) and diluted to volume. Liquid chromatography analyzed these solutions for D–glucose content.

We added second 10.0-mL aliquots of the hydrolyzates to 100-mL volumetric flasks along with 10.0-mL aliquots of a standard solution of D–fructose (20.40 μg/mL) and diluted to volume. We analyzed these solutions for D–xylose and D–mannose contents by liquid chromatography.

Liquid chromatography

All chromatography used a Dionex system (Dionex Corp., Sunnyvale, CA) consisting of a Series 4500i gradient pump and systems controller, an eluant degas module, an advanced chromatography module injection system (50-μL loop), a CarboPac PA Guard column, a Carbopac PA1 (HPIC-AS6-10μ) anion exchange column, and a PAD. **Table II** shows the PAD settings. The output range was 3 k. The eluant flow rate through the column was 1.0 mL/min unless otherwise noted. A Dionex DQP-1 post-column pump metered a 0.3-*M* aqueous, low carbonate sodium hydroxide solution (0.5 mL/min) into the effluent stream after the column and before the PAD. An attached Dionex 4270 integrator recorded and integrated (attenuation = 1074) the areas under the chromatographic peaks.

Table II indicates the eluant solutions. Analyses for monosaccharide contents used the sequence of operations in Table II unless otherwise noted. All solutions used a minimum of three injections.

In the case of the analysis for D–glucose only the total monosaccharide content was low. Accordingly there was a new injection every 20 min. When peak separation diminished (six or more samples), we repeated the complete cycle of column regeneration.

Instrument calibration equations

Liquid chromatography allowed analysis of five standard solutions, each containing authentic L–fucose (6.33 μg/mL) and a different amount of authentic D–glucose (3.99, 5.97, 7.97, 9.97, or 11.96 μg/mL). Using the method of least squares (Sigma Plot Scientific Graph System, Jandel Scientific, Corte Madera, CA), it was possible to treat mathematically the weight ratios and corresponding peak area ratios.

We used liquid chromatography for analysis of an additional five standard solutions. Each of these contained authentic L–fucose (1.81 μg/mL), authentic D–fructose (3.06 μg/mL), and authentic D–glucose (13.80 μg/mL).

Each also contained a different amount of authentic D–xylose (1.05, 1.58, 2.10, 2.62, or 3.15 μg/mL), authentic D–mannose (1.00, 1.50, 2.00, or 3.00 μg/mL), authentic D–galactose (1.00, 1.50, 2.00, 2.50, or 3.00 μg/mL), and authentic L–arabinose (1.02, 1.53, 2.04, 2.54, or 3.05 μg/mL). The method of least squares permitted a mathematical analysis of the calculated weight ratios of D–xylose and D–mannose to D–fructose and their corresponding peak area ratios.

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