Rapid carbohydrate analysis of wood pulps by ion chromatography

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ABSTRACT: Ion chromatography provides a rapid method to analyze carbohydrates in woods and pulps. With optimum conditions, carbohydrate analysis takes 20 min including cleaning and reconditioning of the column. The method allows analysis of 72 samples in 24 h. The authors include the necessary chromatographic conditions for the rapid analysis.

KEYWORDS: Analysis, carbohydrates, chromatography, hydrolysis, hydrolyzates, ion exchange, monosaccharides, optimization, pulps, sampling, wood, yield.

TAPPI Test Method T 249 cm-85 or other equivalent methods have been the accepted procedures for analysis of wood and pulp carbohydrates. The TAPPI method involves neutralization of the acid hydrolyzate with $Ba(OH)_2$, addition of inositol as internal standard, and reduction with $NaBH_4$. The next step is acetylation of the resulting monosaccharides, alditols, and inositol with acetic anhydride using sulfuric acid as catalyst. Finally, there is extraction of alditol acetates with methylene chloride and quantitative determination by gas chromatography. The technique is long because it involves hydrolysis, acetylation, and other complicated processes. A few days are therefore necessary to complete the carbohydrate analysis of a sample.

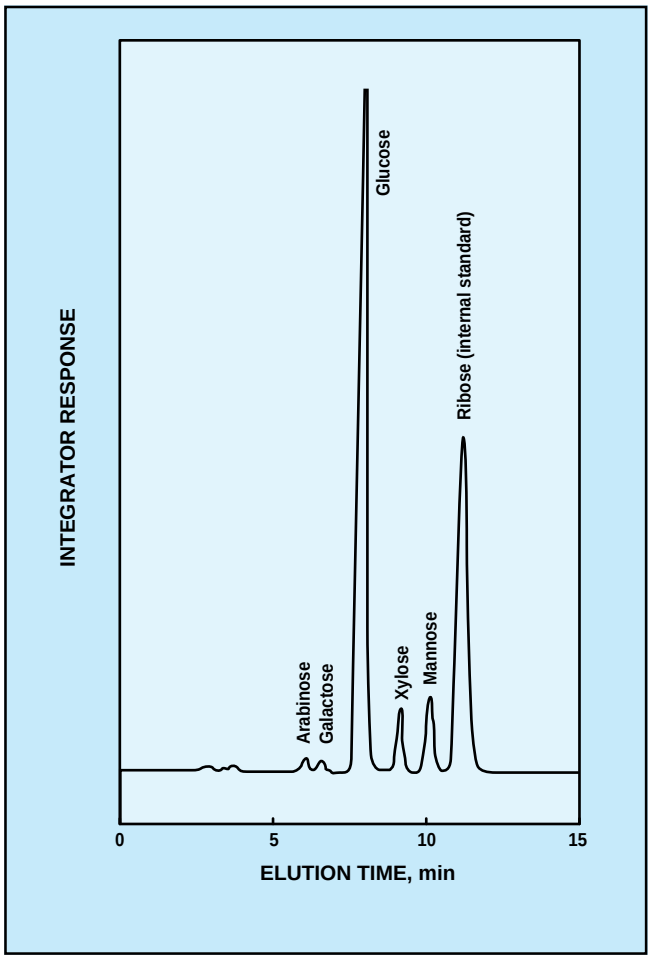
It is possible to incorporate recent progress in the development of high-performance liquid chromatography (HPLC) techniques to the carbohydrate analysis of woods and pulps. Because no derivatization of monosaccharides is necessary, the determination of carbohydrates in samples requires less time for sample preparation. Chromatography also allows the measurement of small amounts of monosaccharides. Woods and pulps usually contain small quantities of arabinose and galactose with a large amount of glucose. This mixture makes it very difficult to determine the minor components correctly. Determination of carbohydrates by HPLC using an ion chromatographic method is a simple technique to analyze carbohydrates in samples. The additional use of pulsed amperometric detector (PAD) provides a highly sensitive detection of monosaccharides.

Using an ion chromatographic technique, Hardy and coworkers[1] determined carbohydrates in glycoproteins in 15 min. The separation of xylose from glucose was not satisfactory, however. Petterson and Schwandt[2] applied a similar method to determine carbohydrates in wood pulps. Their analysis of one sample required approximately 75 min. This included the cleaning and conditioning of the column. The recent work of Laver and Wilson[3] reported the successful analysis of hydrolyzates from rayons and a dissolving pulp using an ion chromatographic technique with an amperometric detector. These investigators reported a determination of the major component—glucose—within 20 min. This time did not include the reconditioning of the column that would be necessary after several sample injections. Minor components may require an analysis time as long as 60 min. This paper will provide convenient conditions for rapid carbohydrate analysis of woods and pulps by ion chromatography.

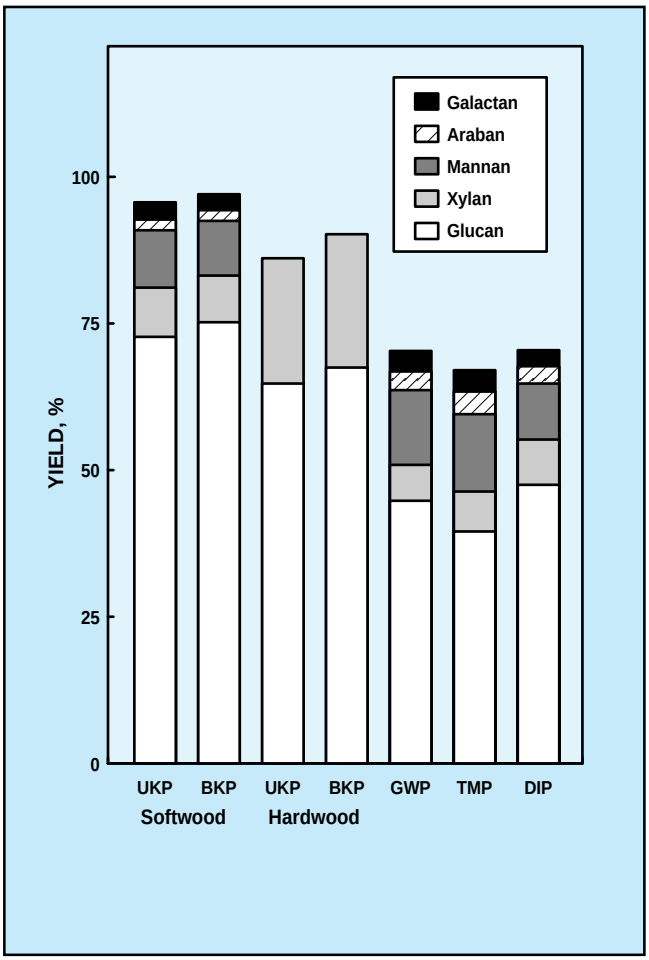
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1. Chromatogram of hydrolyzate from acid-catalyzed hydrolysis of unbleached softwood kraft pulp



2. Composition of monosaccharides in hydrolyzates and total carbohydrate contents of wood pulps



Results and discussion

Optimization of conditions

A strong anion-exchange column separates the monosaccharides that form carbohydrates in pulp. The monosaccharides elute in the order arabinose, galactose, glucose, xylose, and mannose. Separation of these monosaccharides is according to the differences in their dissociation constants. The column shows good baseline stability. This is a key characteristic during the gradient operation.

Because of the small diffusion of the sample components into the column packing material, the chromatographic trace returned to the baseline after each peak. It was possible to determine small amounts of monosaccharides because of the excellent signal to noise ratio. The elution time was approximately 30 min with an extra 10 min for column conditioning in the early stages of this study. Operating conditions were as follows:

- Distilled water conditioned the column for 10 min.
- Immediately after injection of a sample solution, we changed the eluent gradually to 3 *mN* NaOH in 25 min—1 min with 0.3 *N* NaOH and then 4 min with H_2O. Flow rate was 1 mL/min throughout this study.

To assure rapid analyses with good reproducibility, it was necessary to shorten both the elution time and the column conditioning time.

Increasing the alkalinity of the eluent shortened the elution time, but the separation and the reproducibility suffered. Adding a column cleaning step with 0.2 *N* KOH and then introducing a proper amount of carbonate anion onto the column remarkably improved both the separation and reproducibility. The elution time and the column reconditioning time were considerably shorter. The treatment with KOH solution effectively prevents accumulation of carbonate anion on the column. The subsequent introduction of a controlled amount of adsorbed carbonate anion effectively minimizes variation in the elution time for each monosaccharide. Following are the improved column operating conditions:

- Distilled water conditions the column for 5 min.
- After injection of the sample solution, the water flow continues for 10 min.
- The column undergoes elution with 0.3 *N* NaOH for 1 min, with 0.2 *N* KOH for 0.5 min, with distilled water for 0.3 min, with 0.1 *N* Na_2CO_3 for 0.1 min, and again with distilled water for 3.1 min.

There was also an investigation on the optimum alkaline solution for post column washing to assure good sensitivity for detecting reducing monosaccharides. The optimum solution was 0.5 *N* NaOH. Higher concentrations of alkaline solutions caused clogging of the narrow PTFE damper tube because of the deposition of crystalline materials.

Figure 1 shows a typical chromatogram of hydrolyzate from an unbleached softwood kraft pulp. Using our improved method, it was possible to shorten the elution time to 15 min. It was also possible to reduce the time required for reconditioning of the column. We could therefore analyze one hydrolyzate sample every 20 min or 72 samples in 24 h. We have analyzed approximately 10,000 samples using the same column.

Figure 2 shows the monosaccharide composition of hydrolyzates prepared from seven wood pulp samples under the same reaction conditions. The total carbohydrate contents in unbleached groundwood pulp (GWP) and thermomechanical pulp (TMP) are approximately 70% on o.d. pulp. The unbleached GWP and TMP contain almost the same amount of lignin as the corresponding wood chips. Because softwoods contain approximately 30% lignin, the total carbohydrate contents in these pulps cannot exceed 70% o.d. pulp. Since newsprint consists mainly of mechanical pulp and deinked pulp (DIP) comes from old newspaper, they have compositions similar to GWP and TMP.

Unbleached softwood and hardwood kraft pulps (UKP) of bleach-grade and the corresponding bleached kraft pulps (BKP) have low lignin content. These pulps therefore gave higher total carbohydrate yields than the mechanical pulps. The amounts of residual lignin in softwood and hardwood UKP are approximately 4.5% (kappa no. 30 for softwood UKP) and 3% (kappa no. 20 for hardwood UKP), respectively. The total carbohydrate contents in these pulps are approximately 95% on o.d. pulp. Since BKP is almost free from lignin, the total carbohydrate content is close to 100% on o.d. pulp. As Fig. 2 illustrates, the total carbohydrate contents in the UKP and BKP agree well with the expected values.

Hardwood UKP and BKP, however, contained almost 10% less total carbohydrate content than the expected values. Not optimizing the reaction conditions for hydrolysis could cause this. The total yields of monosaccharides are therefore not the optimal values. A low total carbohydrate yield results either from further degradation of monosaccharide produced from insufficient hydrolysis of carbohydrates or from the combination of both factors under the reaction conditions. In this experiment, the reaction conditions for hydrolysis of pulps were mild to prevent further degradation of the monosaccharides produced. Insufficient hydrolysis is therefore the most likely cause of the low total carbohydrate contents determined for hardwood kraft pulps.

Need for rapid analysis

Acid-catalyzed hydrolysis commonly uses specific reaction conditions chosen to obtain optimum monosaccharide yields with a single treatment. As described above, however, the reaction conditions employed for the hydrolysis of pulps are insufficient to convert the carbohydrate completely to corresponding monosaccharides. This subsequently leads to incorrect carbohydrate composition. It is therefore probable that appreciable amounts of oligosaccharide result from the hydrolysis of pulps under the mild reaction conditions. This would give unexpectedly lower total carbohydrate contents in the pulps.

To optimize the conversion of carbohydrates into monosaccharides, one must run several experiments for hydrolysis of a pulp under several sets of reaction conditions. Such experiments will determine reaction conditions that will produce each monosaccharide in optimum yield. It is also desirable to conduct the hydrolysis in duplicate under the same reaction conditions to confirm the accuracy of the original data. Consequently, several hydrolyses of a pulp sample are necessary followed by analysis of the resulting hydrolyzates. To attain this goal, a simple and rapid analytical method for the determination of monosaccharides in the hydrolyzates is necessary. The method discussed in this paper can provide a basis for developing such analytical methods for determination of monosaccharides from hydrolysis of wood pulps.

The authors have also developed a well-defined procedure for the hydrolysis of pulp samples. They will soon publish the procedure elsewhere with the results from the optimized hydrolysis of pulp samples.

Experimental

Sample preparation and reaction conditions

Pulp samples underwent air drying to a moisture content less than 10% on o.d. pulp. An accurately weighed sample of approximately 110 mg (100 mg on o.d. base) underwent hydrolysis according to TAPPI Test Method T 249 cm-85. After addition of 3 mL of 72% H_2SO_4, the sample conditioned in a water bath at 30°C for 1 h. After pouring the reaction mixture into an autoclavable glass bottle, diluting with distilled water to 2% H_2SO_4, and capping tightly, we heated it in an

autoclave for 1 h at 120°C. The low acid concentration prevented further degradation of the resulting monosaccharides. From the hydrolysis of the sample to the determination of the monosaccharides in the resulting hydrolyzate, we performed the experiment in duplicate to check the accuracy of the original data.

After cooling the hydrolyzate in ice water, we filtered it to remove any solid mass. Then we injected the filtrate directly into an ion chromatograph (Dionex Corp.) to analyze the carbohydrate composition. An automated sampler (Dionex Corp.) automatically conducted the filtration and the injection enabling us to do sequential analysis.

Analytical conditions

The experimental work used a Dionex model 2020i ion chromatograph equipped with a PAD. A delivery pump (DQP-1, Dionex Corp.) supplied the alkaline solution for post column washing. The analyzing column was a CarboPac™PA-l column (Dionex Corp., 4 mm x 250 mm) with a short guard column of the same material. Packing for the columns was a styrene-divinylbenzene copolymer with 10 μm diameter coated with anion latex with 0.2 μm diameter. The detector had gold acting electrodes. Voltage change was cyclical as follows: E1 (sampling) = 0.1 V (300 ms), E2 (plate cleaning) = 0.60 V (120 ms), and E3 (plate activating) = -0.80 V (60 ms).

The eluent for sugar separation was pure water. Since adsorbed carbonate ion on the column packing material shortens the elution times of monosaccharides, deionized and distilled water underwent heating to 40°C under reduced pressure with moderate stirring to remove dissolved CO_2. We placed this water in a plastic tank and continuously bubbled helium gas through it to prevent CO_2 from entering. This was the same water for preparing the alkaline solutions.

Data accumulation and determination of sugars

To standardize the analysis, we used solutions consisting of monosaccharides having compositions similar to those in the hydrolyzates from wood pulp. Analyzing a solution consisting of monosaccharides of known composition after every tenth unknown sample analysis allowed us to check fluctuation in the sensitivity. By monitoring changes in the peak height caused by addition of newly prepared eluent or unexpected chemical or biochemical degradation of the samples, it was possible to assure consistency in the analysis.

A Labchart 180 (System Instruments Co.) controlled the system and data acquisition. We discarded a few injections at the beginning of every continuous analysis to condition the column. Because carbohydrates in pulp have a degree of polymerization over 20, we calculated all the monosaccharides determined as anhydrous derivatives.

Conclusion

This work optimized the operating conditions for analysis of hydrolyzates from acid-catalyzed hydrolysis of wood pulps by ion chromatography. By adsorbing an appropriate amount of carbonate ion on an anion exchange column, it was possible to determine monosaccharides in the hydrolyzate samples in 15 min. Sequential analyses that take only 20 min for analysis of one sample are therefore feasible. This analysis time includes the conditioning time of the column. With this method, one can analyze 72 hydrolyzates in 24 h.

Using our conditions, we compared the total monosaccharide yields from acid-catalyzed hydrolysis of seven pulp samples. The total yields agreed well with the expected values except hardwood bleached and unbleached kraft pulps. These results indicate that the optimum reaction conditions for acid-catalyzed hydrolysis of pulps differ from sample to sample. It is also conceivable that the optimum reaction conditions suitable for one carbohydrate component do not give the optimum yield for another component in the same sample. It is therefore necessary to optimize the reaction conditions for acid-catalyzed hydrolysis of carbohydrates in pulp samples. The operating conditions described here meet these needs. TJ

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