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Note

Separation of wood degradation products by high-performance liquid chromatography

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Due to the interest in biomass, *i.e.*, wood degradation products, as a new source both of chemical raw materials and potent energy carriers, the analysis of hydrolysis products resulting from acidic, hydrothermal or enzymatic treatment is of increasing importance[1–7]. Compounds resulting from hydrolysis are oligomeric carbohydrates, cellobiose, glucose, xylose as well as products of further degradation such as dihydroxyacetone, glyceraldehyde, methylglyxoal, acids, hydroxymethylfurfural (HMF) and furfural.

High-performance liquid chromatography (HPLC) has generally replaced the time-consuming gas chromatographic (GC) methods[8,9] for the separation of compounds formed by biomass degradation. The determination of sugars is often performed with columns containing amino-bonded materials, which show good separation characteristics for monomeric and oligomeric carbohydrates[10,11]. For the analysis of degradation products, such as HMF and furfural, other column materials *e.g.*, C_8 and C_{18}, are employed[12,13]. Recently HPLC with ion-exchange materials and organic resins has become increasingly important[14–19].

The aim of the present work was a comparison of column packing materials suitable for the analysis of the products obtained upon acidic, hydrothermal and enzymatic hydrolysis.

EXPERIMENTAL

Apparatus

A high-performance liquid chromatograph (Model SP 8000 B; Spectra Physics, Santa Clara, CA, U.S.A.), with an integrated data system and column oven compartment, was employed with a differential refractive index detector (Model R 401; Waters Assoc., Milford, MA, U.S.A.). The samples were injected by a valve fitted with a 25-μl loop (Valco Instruments Co., Houston, TX, U.S.A.). The eluents were degassed with helium during chromatography.

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Columns

The following prepacked columns were employed: HPX-87H and HPX-42A (300 × 7.8 mm I.D.; Bio-Rad Labs., Richmond, CA, U.S.A.) with ion-exclusion micro-guard cartridges. For the analysis on μSpherogel-Carbohydrate × 7.5 (300 × 7.5 mm I.D.; Beckman Instruments Inc., Berkeley, CA, U.S.A.) a precolumn was also necessary. A combination of three TSK PW 2000 columns and one TSK PW 3000 (all 300 × 7.5 mm I.D., Beckman Instruments) with a TSK PW precolumn was employed for further examinations. The chromatographic conditions for each column system are given in the figure captions.

Samples

Poplar wood and filter-paper were degraded hydrothermally[20]. The solutions obtained were concentrated using a reverse osmosis system, filtered with a membrane filter (0.2 μm, Schleicher & Schüll) and analyzed. All reference standard solutions were prepared from analytical grade chemicals (Fluka, Buchs, Switzerland; Merck, Darmstadt, F.R.G.).

RESULTS AND DISCUSSION

We investigated the analysis of reference mixtures, and of solutions which were obtained by hydrolyzing either lignocellulose-containing phytomass, *e.g.*, wood, or cellulose-containing material, *e.g.*, filter-paper.

Sugars and their degradation products

The main hydrolysis products of cellulose- and hemicellulose-containing biomass are sugars. In addition there is always a certain amount of sugar degradation compounds, such as low-molecular-weight aldehydes, ketones and acids. Fig. 1 shows the analysis of a set of twelve reference substances, using an HPX-87H column, refractive index detection and 0.01 *N* sulphuric acid as mobile phase. For these substances the detection limit was below 0.1 mg/ml. Fig. 2 shows the chromatogram of a solution obtained by the hydrothermal degradation of cellulose (filter-paper), using an HPX-87H column. A rapid routine analysis of hydrolyzates can be achieved, since only filtration of the solutions is necessary before analysis. The same column packing material was also used for the analysis of fermentation solutions. Fig. 3 shows the results of an ethanol fermentation of a solution prepared by hydrothermolysis of filter-paper.

For the determination of the different hexoses and pentoses obtained upon hydrolysis, a μSpherogel column was used. Fig. 4 shows the chromatogram of a hydrothermally degraded cellulose (filter-paper). The first peak is due to higher oligomeric sugars. The cellobiose, glucose and its isomerization products (fructose and mannose) as well as the further degradation product hydroxymethylfurfural are separated. Fig. 5 shows the analysis of hydrothermally degraded poplar wood (populus tremuloides). In addition to the compounds resolved in Fig. 4, xylose also appears which originates from the hemicellulose part of the wood material.

Gluco-oligomers

The determination of gluco-oligomeric compounds in non-acidic biomass hy-

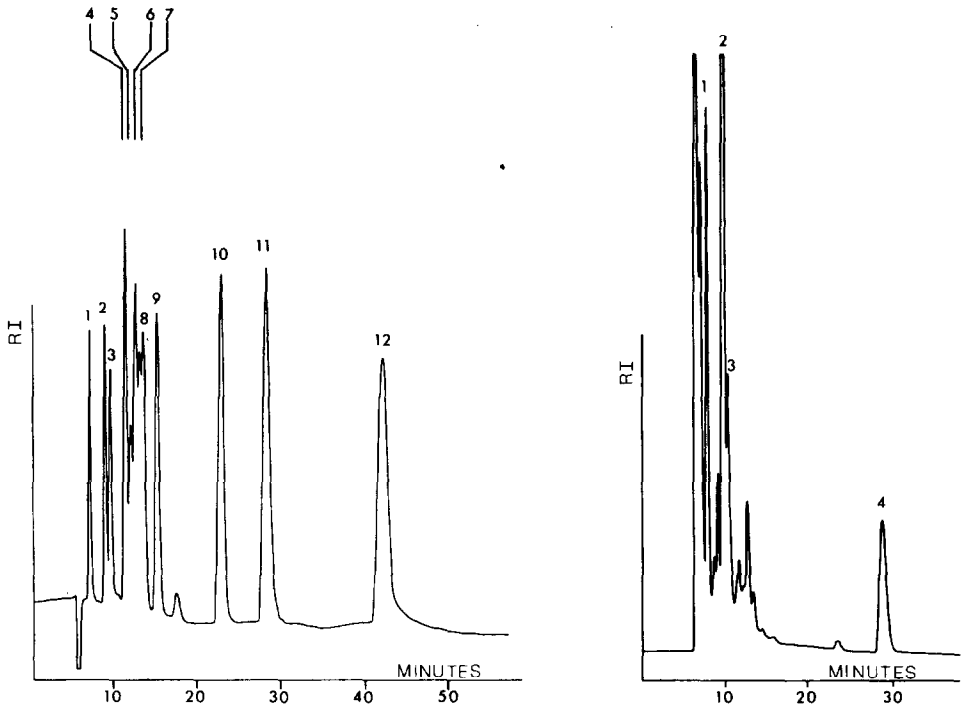

Fig. 1. HPLC chromatogram of a standard solution. Conditions: HPX-87H + ion exclusion micro-guard (cation H); mobile phase, 0.01 *N* sulphuric acid; flow-rate, 0.6 ml/min; column temperature 70°C; detection refractive index (RI). Peaks: 1 = cellobiose; 2 = glucose; 3 = fructose; 4 = glyceraldehyde; 5 = methylglyoxal; 6 = glycoaldehyde; 7 = dihydroxyacetone; 8 = formic acid; 9 = levulinic acid; 10 = ethanol; 11 = HMF; 12 = furfural.

Fig. 2. HPLC chromatogram of a hydrothermal degradation of filter-paper. Conditions as in Fig. 1. Peaks: 1 = cellobiose; 2 = glucose; 3 = fructose; 4 = HMF.

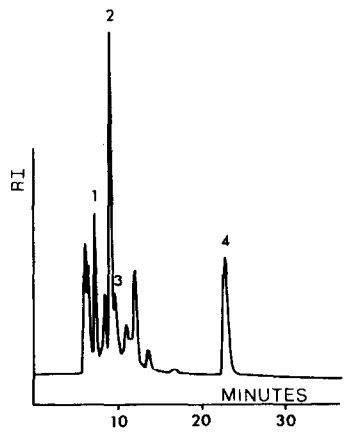

Fig. 3. HPLC chromatogram of a fermentation solution of a hydrothermally degraded filter-paper (extracted with ethyl acetate). Conditions as in Fig. 1. Peaks: 1 = cellobiose; 2 = glucose; 3 = fructose; 4 = ethanol.

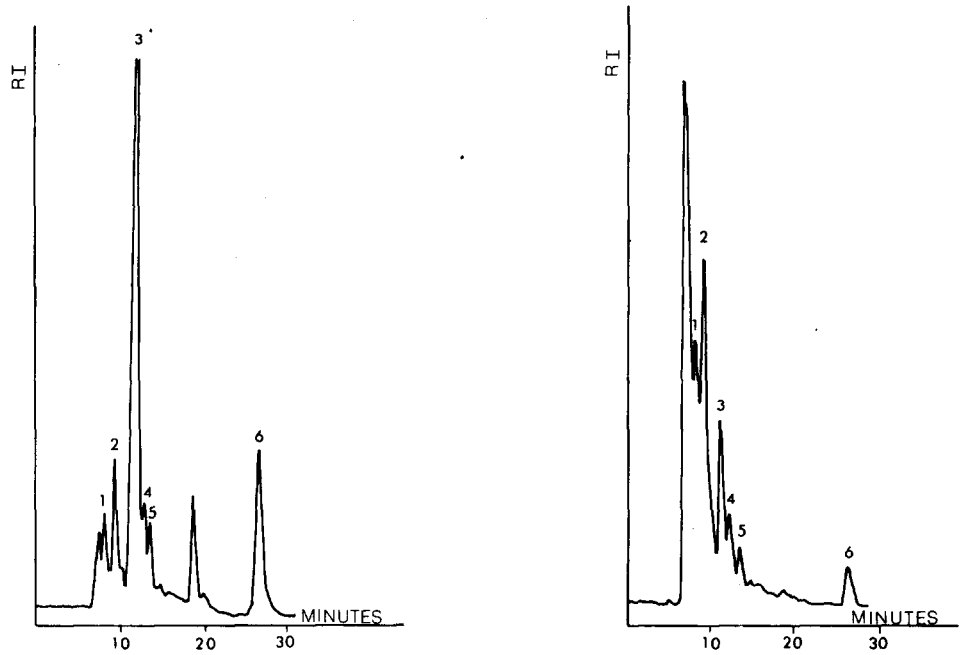

Fig. 4. HPLC chromatogram of a hydrothermal degradation of filter-paper. Conditions: μSpherogel × 7.5 Carbohydrate + ion exclusion micro-guard (anion OH); mobile phase, water; flow-rate, 0.6 ml/min; column temperature, 85°C; detection, RI. Peaks: 1 = oligomers; 2 = cellobiose; 3 = glucose; 4 = mannose; 5 = fructose; 6 = HMF.

Fig. 5. HPLC chromatogram of a hydrothermal degradation of poplar wood. Conditions as in Fig. 4. Peaks: 1 = oligomers; 2 = cellobiose; 3 = glucose; 4 = xylose; 5 = fructose; 6 = HMF.

drolysates was studied by use of an HPX-42A column. A gluco-oligomeric hydrothermal degradation solution, containing oligomers up to degree of polymerization (DP) 8, can be evaluated within 25 min (Fig. 6).

Due to the fact that the hydrothermal degradation can proceed without acidic and alkaline catalysts, the above column is especially suitable for the determination of oligomers in hydrothermal reaction solutions. Compared with gel chromatography, *e.g.*, Bio-Gel P[21–23], the HPX-42A column has the advantage of greatly shortening the analysis time. It is also preferable to amino-bonded packing materials because water can be used as mobile phase, resulting in higher sensitivity of refractive index detection. For the determination of solutions obtained by acidic or alkaline hydrolysis, the samples have to be prepared with the aid of an auxiliary ion-exchange material.

Another stationary phase suitable for the separation of oligomers in acidic hydrolysates is TSK PW. This organic resin has the advantage of high stability both over a wide pH range (2–12) and towards ionic influences. Therefore its field of application encompasses both acidic and alkaline biomass degradation solutions. If salt solutions are used as eluents, intermolecular interactions can be reduced so as to allow a separation of ionic substances. The only preliminary treatment necessary is

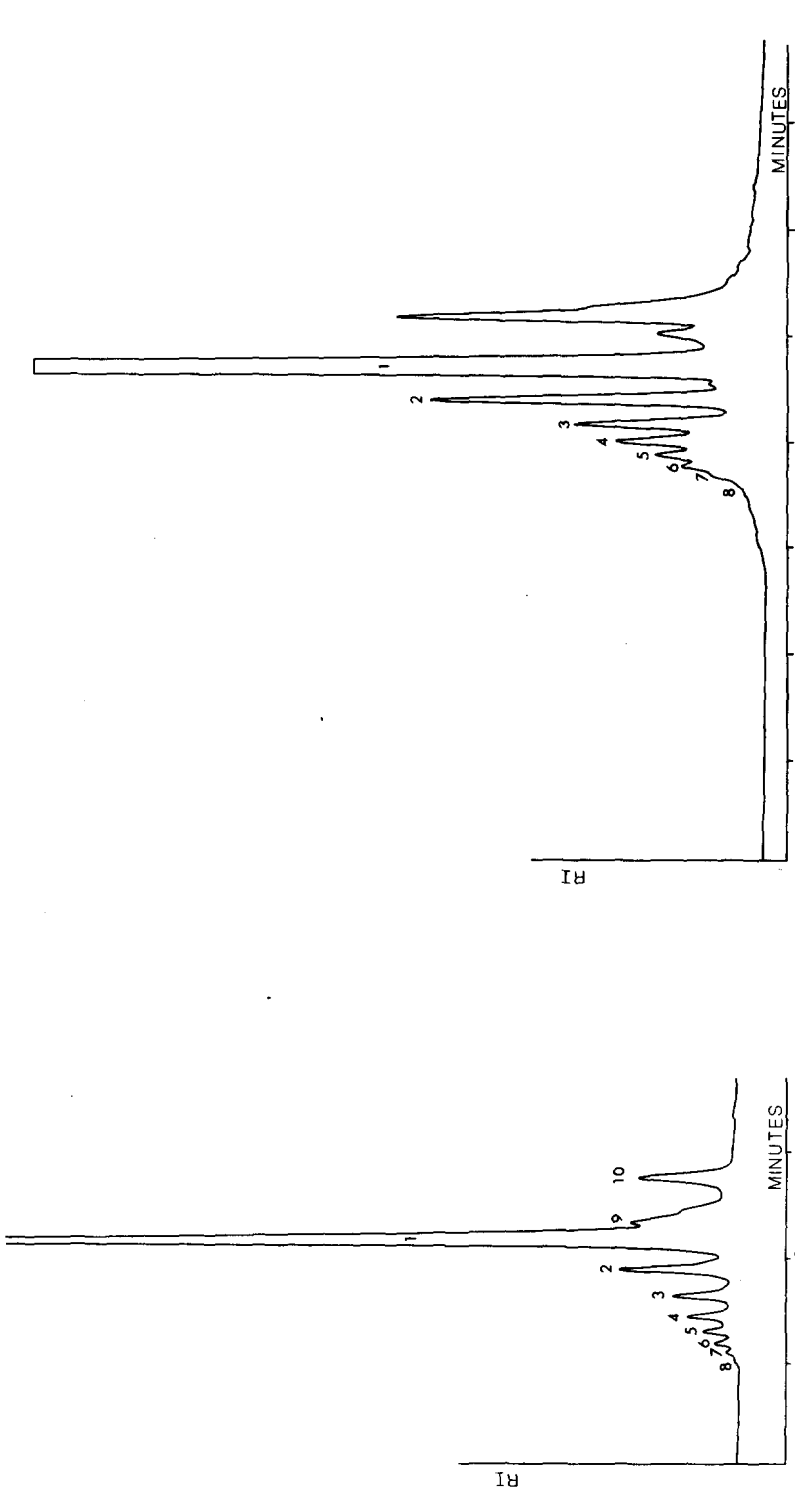

Fig. 6. HPLC chromatogram of gluco-oligomers of hydrothermally degraded poplar wood (populus tremuloides). Conditions: HPX-42A and ion exclusion micro-guard (anion OH); mobile phase, water; flow-rate, 0.5 ml/min; column temperature, 85°C; detection, RI. Peaks: 1 = glucose; 2 = cellobiose; 3–8 = numbers indicate DP; 9 = fructose; 10 = further degradation products.

Fig. 7. HPLC chromatogram of gluco-oligomers of hydrothermally degraded filter-paper. Conditions: TSK PW 2000 (3 ×) and TSK PW 3000 (1 ×) with TSK PW precolumn; mobile phase, water; flow-rate, 0.8 ml/min; column temperature, 50°C; detection, RI. Peaks: 1 = glucose; 2 = cellobiose; 3–8 = numbers indicate DP.

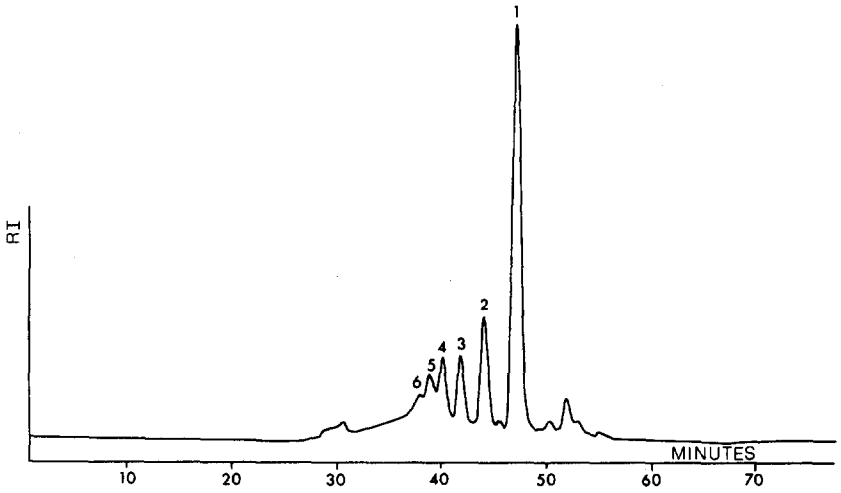

Fig. 8. HPLC chromatogram of gluco-oligomers of hydrothermally degraded poplar wood. Conditions as in Fig. 7. Peaks: 1 = glucose; 2 = cellobiose; 3–6 = numbers indicate DP.

the application of a C_{18} cartridge filter to remove lignin components. In order to obtain good separations of sugars over a large range of molecular weights (including DP 1), several columns can be connected in series, requiring a longer analysis time. With a combination of TSK PW columns, oligomers from hydrothermally degraded filter-paper and poplar wood are well separated (Figs. 7 and 8).

TABLE I

COMPARISON OF PACKING MATERIALS FOR THE HPLC DETERMINATION OF GLUCO-OLIGOMERS, MONOSACCHARIDES AND THEIR DEGRADATION PRODUCTS

Key: ++ = good resolution; + = long retention time; – = other compounds of the standard solution interfere; –– = unusable.

Biomass compounds	*HPX-87H*	*HPX-42A*	*μSpherogel 7.5 carbohydrate*	*TSK PW 2000 3×, TSK PW 3000 1×*
Oligomeric sugars	––	++	––	++
Cellobiose	++	+	++	+
Glucose	++	+	++	+
Fructose	–	––	++	––
Mannose	–	––	++	––
Xylose	–	––	++	––
Glyceraldehyde	++	––	–	––
Methylglyoxal	++	––	–	––
Glycolaldehyde	++	––	–	––
Dihydroxyacetone	++	––	–	––
Formic acid	++	––	––	––
Levulinic acid	++	––	––	––
Ethanol	++	––	++	––
Hydroxymethyl-furfural (HMF)	+	––	+	––
Furfural	+	––	+	––
Oligomeric sugars	––	++	––	++

In order to control the degree of degradation and to study the process of biomass hydrolysis, the analysis of monomeric and oligomeric water-soluble carbohydrates is of especial importance. The TSK PW and HPX-42A columns enable the determination not only of carbohydrate compounds up to the monomeric sugars, but also of the molecular weight distribution in each fraction of the hydrolysate. Therefore a complete record of the spectrum of biomass degradation products is obtained by applying a combination of columns, one to determine the monomeric carbohydrates and their degradation products and another to evaluate the distribution of oligomers. A comparison of the examined columns for the separation of biomass hydrolysis products is given in Table I.

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