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CHROMATOGRAPHIC ANALYSIS OF BIOMASS REACTION PRODUCTS PRODUCED BY HYDROTHERMOLYSIS OF POPLAR WOOD

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SUMMARY

Poplar wood was treated under hydrothermal conditions at various temperatures and the wide spectrum of reaction products was analysed by different liquid chromatographic methods. Low-molecular-weight compounds were investigated by means of high-performance liquid chromatography. Sugars and sugar decomposition products were analysed using a cation-exchange column; the separation of lignin degradation products was carried out by reversed-phase chromatography with gradient elution. The high-molecular-weight components were characterized by gel permeation chromatography (GPC). Oligomeric sugars were eluted from a gel column with water as the eluent. The molecular weight distribution of degraded lignin was obtained by GPC with a mixture of dioxane–water as the eluent. The application of the methods described does not require a time-consuming sample preparation. Therefore a rapid analysis and quantification of the reaction products is possible. This allows the investigation of the course of reaction and the calculation of the mass balance. In addition, the optimum process parameters can be evaluated from kinetic studies of the hydrothermolysis reaction.

INTRODUCTION

The biosphere contains vast amounts of renewable sources for a number of raw materials and chemicals, whereby the main portion of the naturally occurring carbon compounds are found in plant matter. However, biomass does not show an uniform composition, but consists of low-molecular-weight compounds and an high-molecular-weight framework, which is responsible for the rigidity of plant matter. The low-molecular-weight part is built from inorganic material (ash content) and organic compounds, of which the latter can easily be extracted. The high-molecular-weight part shows a complex composition and consists of three main components, two of which are polysaccharides, cellulose and hemicellulose; the third component is lignin, which is polyaromatic and has a cross-linked structure.

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From this heterogenic character of biomass it can readily be concluded that chemical conversion and utilization by processes such as saccharification, gasification, pyrolysis, hydrothermolysis, liquefaction, hydrogenation, etc., lead to a wide spectrum of compounds and degradation substances. In most cases, the reactions of the polysaccharides result in the formation of oligosaccharides, monomeric sugars and their degradation products. Chemical decomposition of lignin yields substances which can be extracted with organic solvents; some of them are phenol carbonic acids, phenols, aromatic aldehydes and ketones, as well as different condensation products.

In order to effect analysis of the diverse products formed by the chemical conversion of biomass, versatile procedures are required. By means of liquid chromatography a rapid determination of the most important products can be achieved. The chemical properties of the reaction products derived from the polysaccharides and the polyaromatic constituent respectively are very different. For this reason, various separation systems have to be used, which allow the identification of monomeric substances as well as oligomeric components.

The liquid chromatographic (LC) methods described in this work show the range of application of high-performance liquid chromatography (HPLC) and gel permeation chromatography (GPC) to the analysis of biomass degradation solutions prepared by hydrothermolysis of poplar wood. With this process, high-molecular-weight substances from biomass can be degraded in three main steps. First, hemicellulose and a certain amount of lignin are eluted; secondly, cellulose is degraded and thirdly, the residual lignin is decomposed[1–3].

Extractive matter

The determination of low-molecular-weight extractable substances by liquid chromatography is described extensively elsewhere[4–6]. Separation of aromatic and phenolic components, terpenes, flavonoids, aliphatic acids as well as resinic acids, alcohols and sterols is performed using a reversed phase with different gradient elutions, mainly with a buffer–acetonitrile or an alcohol mixture[7–14].

Polysaccharides including sugars and sugar degradation products

Results can be obtained very quickly, without time-consuming sample preparation, by HPLC of monomeric sugars on amino-bonded stationary phases[15–17]. Separation on silica gel with amino modifiers has also been carried out with good results[18–20]. For high-molecular-weight substances, *e.g.*, oligomers, the applicability of these column materials is limited[21–23]. Most substances, sugars as well as sugar decomposition products, can be analysed by means of cation-exchange resins[24–34].

For rapid quantification of products such as 5-hydroxymethylfurfural (HMF) and furfural, reversed phases are employed with mixtures of methanol–water or acetonitrile–water as the eluent[35,36].

The product distribution of oligomeric sugars is obtained using Bio-Gel P-2 and P-4 columns[37,38]. Also cation-exchange resins with certain counter ions[39] and special materials for HPGPC in the aqueous phase[40,41] have proved to be suitable column materials with the advantage of short analysis times.

Chromatographic analysis of the molecular weight distribution of the residual cellulose, which is insoluble in water, can be carried out in two different ways: either

cellulose has to be made soluble by derivatization, *e.g.*, nitration[42,43] or carbanilation[44,45], or a cellulose solvent, *e.g.*, cadoxen[46,47] or an iron-tartrate solution (FeTNa)[48] has to be applied prior to analysis by selected GPC systems.

Lignin degradation products

The wide spectrum of monomeric decomposition products derived from lignin, which is built up from coniferyl alcohol, sinapyl alcohol and *p*-hydroxycinnamic alcohol respectively, is usually separated by reversed-phase chromatography. Elution from RP C_{18} or RP C_8 columns with different solvent gradients of buffer–acetonitrile or buffer–methanol is employed[5–10,49,50].

Since partially degraded lignins are not soluble in many organic solvents, compounds with adequate dissolving power, *e.g.*, dimethyl sulphoxide (DMSO), dimethylformamide (DMF) or dioxane, are examined as solvents for the determination of the molecular weight distribution[51–55]. To avoid adsorption effects and reversed-phase behaviour, the polarity of the eluent, stationary phase and sample has to be taken into consideration[56,57].

It is seen that LC methods can be used for analysis of biomass degradation solutions without complicated sample preparation, and quantification can be achieved without derivatization. Therefore, in the present work, various separation methods were applied to analyse monomolecular as well as oligomeric products originating from hydrothermolysis of the polysaccharides and lignin of poplar wood.

MATERIALS AND METHODS

Hydrothermolysis of poplar wood

The reaction solutions were prepared by means of an hydrothermolysis apparatus described by Bobleter and Binder[1]. The reaction vessel with a volume of *ca.* 6 cm^3 is fitted with an electrical heating unit. Pure water was heated by passage through a preheating unit and then led to the reaction vessel by a high pressure pump. At temperatures between 160 and 300°C, poplar wood chips (unextracted) were degraded hydrothermally and the reaction products were continuously eluted by hot water. The eluent was collected into fractions after passage through an heat exchanger and a control valve, by which the pressure within the system is maintained over the saturation pressure of the percolating water.

HPLC

A compact HPLC system (Model SP8000B; Spectra Physics, Santa Clara, CA, U.S.A.) with an integrated column-oven compartment and data system and a sample injection valve with a 25-μl loop (Valco Instruments, Houston, TX, U.S.A.) was used.

Sugars and their decomposition products were determined by means of a refractive index (RI) detector (Model 156; Beckman Instruments, Berkeley, CA, U.S.A.), and monomeric lignin substances by a variable wavelength UV-detector (Model SP8400; Spectra Physics) at 280 nm. Carbohydrates were chromatographed isocratically on a prepacked Shodex S 801 column (500 mm × 8 mm I.D.; Showa Denko, Tokyo, Japan) with an ion-exclusion micro-guard cartridge and water as the eluent.

For the separation of lignin degradation products, stainless-steel columns (250 mm × 4.6 mm I.D.; Knauer, Oberursel, F.R.G.) were packed with spherical totally porous silica gel (Nucleosil 5 C_{18}; Machery, Nagel & Co., Düren, F.R.G.). The columns were filled by the slurry-packing technique using a double piston pump (Model 100; Altex, Berkeley, CA, U.S.A.) at 65 MPa with a flow-rate of 10 ml/min.

Chromatographic separations were performed by gradient elution with an initial composition of 95% 0.01 *M* phosphate buffer, pH 2.0 (analytical grade potassium dihydrogenphosphate; E. Merck, Darmstadt, F.R.G.) and 5% acetonitrile (analytical grade; E. Merck) at a flow-rate of 0.8 ml/min. The acetonitrile concentration was raised to 50% within 45 min. In order to purge the system, the acetonitrile content was further increased to 70% within 5 min and maintained for 9 min. To re-equilibrate the column, the initial conditions were adjusted by a 1-min step-gradient which was maintained for 10 min before the next injection was made. The column-oven temperature was kept at 50°C.

GPC

The GPC system for the separation of oligomeric sugars consisted of a precision pump (Model 100, Altex), a sample loop valve (Model 201, Altex) with a 500-μl loop and an RI detector (Model 156, Beckman Instruments Inc.). A glass column (200 cm × 1 cm I.D.) made in our Institute, equipped with a column jacket, was packed with Bio-Gel P-2 (minus 400 mesh; Bio-Rad Labs., Richmond, CA, U.S.A.) as stationary phase and operated at elevated temperatures with water as the eluent. The investigations of degraded lignins were carried out with commercially available solvent-resistant columns (SR 10/50, 50 cm × 1 cm I.D.; Pharmacia, Uppsala, Sweden). The column was packed with an LH-20 gel (Pharmacia) swollen in the solvent. Before packing, however, the gel was treated in an ultrasonic bath for *ca.* 10 min to remove air. The solvent, dioxane–water (7:3), was delivered by a reciprocating piston-pump with a pulse dampener (Model 110, Altex) and passed through the column at a flow-rate of 0.3 ml/min. Samples were applied to the column by a sample loop valve (Altex) using a 100-μl loop. The UV absorption at 254 nm and the refractive index were measured continually with a dual detector (No. 103.07, Knauer); the absorption at 280 nm was monitored with a Model 770 detector (Spectra Physics). All eluents were degassed with helium.

Gas chromatography–mass spectrometry (GC–MS)

A Varian MAT 44S GC/MS system (Varian, NJ, U.S.A.) with a quadrupole mass spectrometer and a capillary gas chromatograph was used. The glass capillary column (25 m × 0.25 mm I.D.) was coated with SE 54 (0.25 μm). Helium was used as the carrier gas at a flow-rate of 1.4 ml/min. The splitter/injector was kept at 240°C with a splitting ratio of 1:5. The column temperature was raised from 70 (3 min) to 240°C (5 min) at 5°C/min. The GC–MS interface was an open split coupling maintained at 240°C. The temperature of the ion source was 190°C, the ionizing current was 600 μA and the electron energy was 70 eV. The chromatograms were recorded by total ion monitoring in the range *m/e* 60–400.

The degradation products from the reaction solution were extracted with chloroform. After evaporation, the substances were derivatized and separated as their trimethylsilyl ethers. The silylating agent used, hexamethyldisilazane–trimeth-

ylchlorosilane–pyridine (2:1:7), at 70°C for 1 h, gives good results with regard to reproducibility.

RESULTS AND DISCUSSION

Poplar wood was hydrolysed under hydrothermal conditions between 160 and 300°C at intervals of 20°C. The fractions with the maximum amounts of degradation products were collected and analysed without prior concentration.

Gluco-oligomers, sugars and sugar degradation products

At temperatures below 290°C a fairly large amount of gluco-oligomers is formed during hydrothermolysis. For the analytical determination of these compounds, a GPC system was set up, which had a particularly good resolving power for cellodextrins. The separation of gluco-oligomers up to DP 8 (DP = degree of polymerization) is shown in Fig. 1. The markedly retarded peaks are the degradation products HMF and furfural. Although the analysis time is rather long in comparison with that obtained on cation-exchange resin columns, this column has the advantage of being insensitive to impurities. Furthermore, the stationary phase (Bio-Gel P-2) is inexpensive and stable over a long period. The results obtained by means of this analytical GPC column can be transferred to the preparative scale without decisive loss of efficiency. Therefore the GPC system described not only offers the possibility to obtain a complete mass balance of the hydrothermal degradation of ligno-cellulosic material, but also enables the isolation of gluco-oligomers from a preparative column under low pressure.

Hydrothermolysis also leads to the formation of low-molecular-weight sugars and their degradation products. Within the last few years, numerous publications

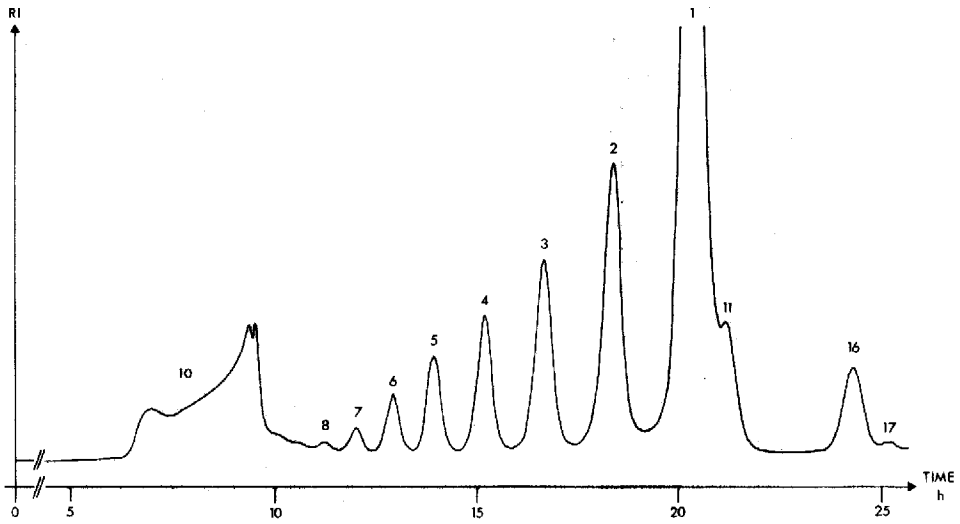

Fig. 1. GPC chromatogram of hydrothermally degraded poplar wood. Conditions: column, Bio-Gel P-2, temperature 50°C; mobile phase, water, flow-rate 0.12 ml/min; detection, RI. Peaks: 1 = glucose; 2 = cellobiose; 3–8 = cellodextrins; 10 = polymers; 11 = xylose/fructose; 16 = HMF; 17 = furfural.

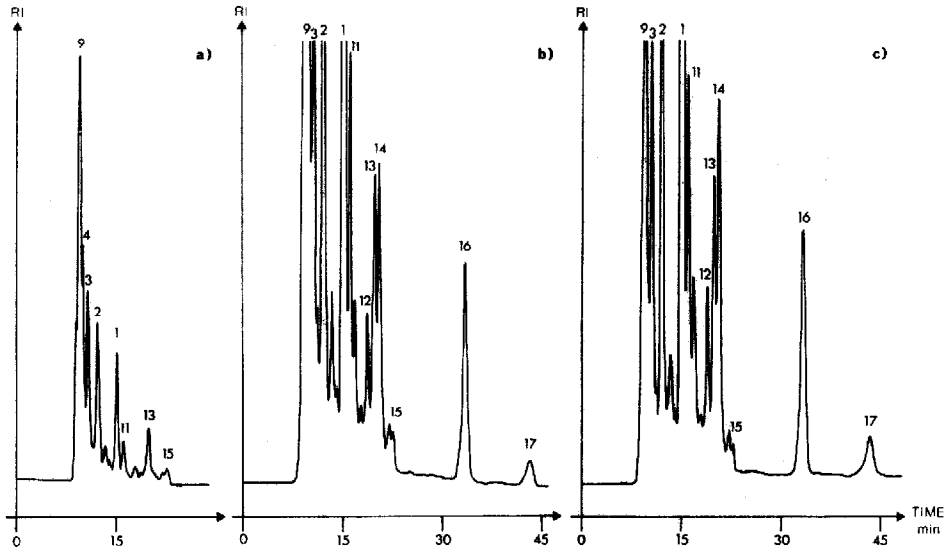

Fig. 2. HPLC chromatograms of three consecutive fractions from hydrothermal degradation of poplar wood. (a) Fraction 5; (b) fraction 6; (c) fraction 7. Conditions: column, Shodex S 801, temperature 85°C; mobile phase, water, flow-rate 0.8 ml/min; detection, RI. Peaks: 1 = glucose; 2 = cellobiose; 3 = cellotriose; 9 = oligomers; 11 = xylose/fructose; 12 = glyceraldehyde; 13 = methylglyoxal; 14 = anhydroglucose; 15 = dihydroxyacetone; 16 = HMF; 17 = furfural.

have dealt with sugar analysis on columns containing ion-exchange resins in the forms H^+, Ca^{2+}, Ag^+ and Pb^{2+} (refs. 33, 34). In this work an HPLC column, packed with a cation-exchange resin with Na^+ as counter ion, was applied. Chromatograms obtained of three consecutive fractions of the hydrothermolysis of poplar wood are shown in Fig. 2. The most important reaction products are separated within a relatively short time. The predominant size-exclusion effect of this column[29] allows the separation of cellotriose and cellotetraose (Fig. 2a), but as a consequence the resolution of monomeric sugars, such as fructose and xylose, is not achieved under the chosen conditions. On the other hand, this column allows a very efficient separation of various degradation products important for calculating the mass balance of this process. In addition, the course of the reaction can be studied. In fraction 5 (Fig. 2a) only a small amount of glucose is formed and almost no degradation products are present. This situation changes drastically in fractions 6 and 7 (Fig. 2b and c), where very high concentrations of sugars and degradation products are formed. After establishing calibration curves for the most important reaction products, quantitative analysis showed that, in this experiment, the concentration, *e.g.*, of glucose reached 4% in the main fraction. This fact is of great importance when further conversion of glucose into ethanol is intended.

Lignin and lignin degradation products

Conventional investigation of the solid residue of poplar wood after hydrothermal degradation shows that lignin is partly decomposed and converted into low-molecular-weight products[1]. The fractions collected are turbid solutions, in which a precipitate is formed after storage for a certain time. This precipitate has a negative influence on the LC analysis. Therefore, in the case of RP chromatography,

acetonitrile–water (1:1, v/v) is added, which results in the formation of a clear solution. In GPC analyses the addition of dioxane–water (1:1, v/v) also effects the formation of clear solutions. This means that tedious sample preparation, *e.g.*, filtration is not necessary prior to injection of a sample on the column.

Monomeric and oligomeric lignin components are extremely reactive substances. They are able to condense to products of high molecular weight at room temperature within a very short time. Fig. 3 demonstrates the gel chromatograms of degradation solutions (after hydrothermolysis at 200°C) obtained after different periods of storage. As a result of the condensation reaction, a clearly visible shift in molecular weight can be observed. Thereby it is demonstrated that the hydrothermolysis products of lignin are very unstable. For this reason, the investigation and analysis of the single compounds present in the solutions have to be performed immediately after hydrothermal treatment. Additional investigations by means of GPC showed that the observed increase in molecular weight occurs to a lesser extent when the degradation solutions are stored at low temperatures or in a freezer. This behaviour was also confirmed by analysis of the monomeric components, which were investigated as a function of the storage time and temperature.

For the degradation experiments, poplar wood shavings without bark were applied. As mentioned, the wood was used in an unextracted form because the extractable percentage was low, 1.44% with benzene–ethanol (9:1, v/v). HPLC analysis is best suited to the investigation of monomeric compounds. The oligomeric part

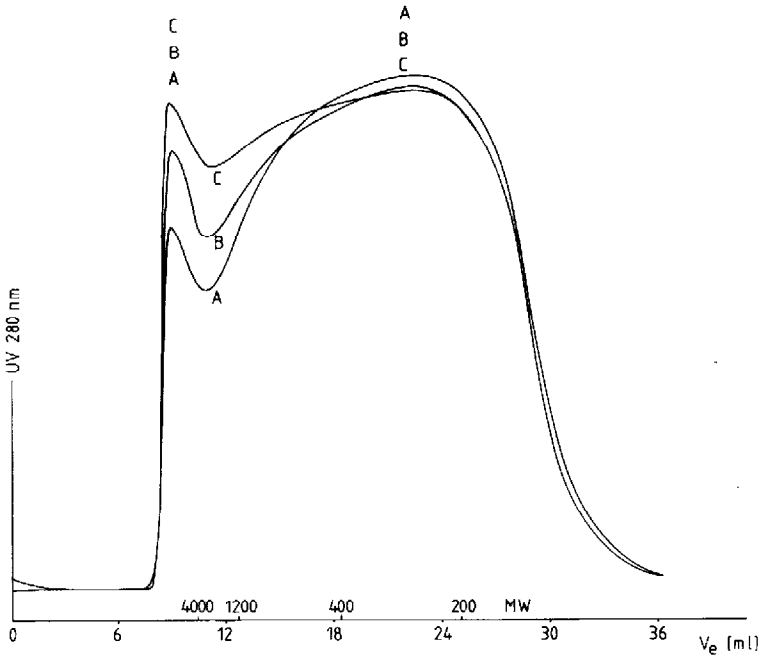

Fig. 3. GPC separation of solutions from hydrothermally treated poplar wood; elution curves of a degradation solution stored at room temperature given as a function of the storage time. GPC column: LH-20 gel, gel bed volume 30.4 ml. (A) 2 h, (B) 24 h, (C) 96 h after hydrothermolysis at 200°C.

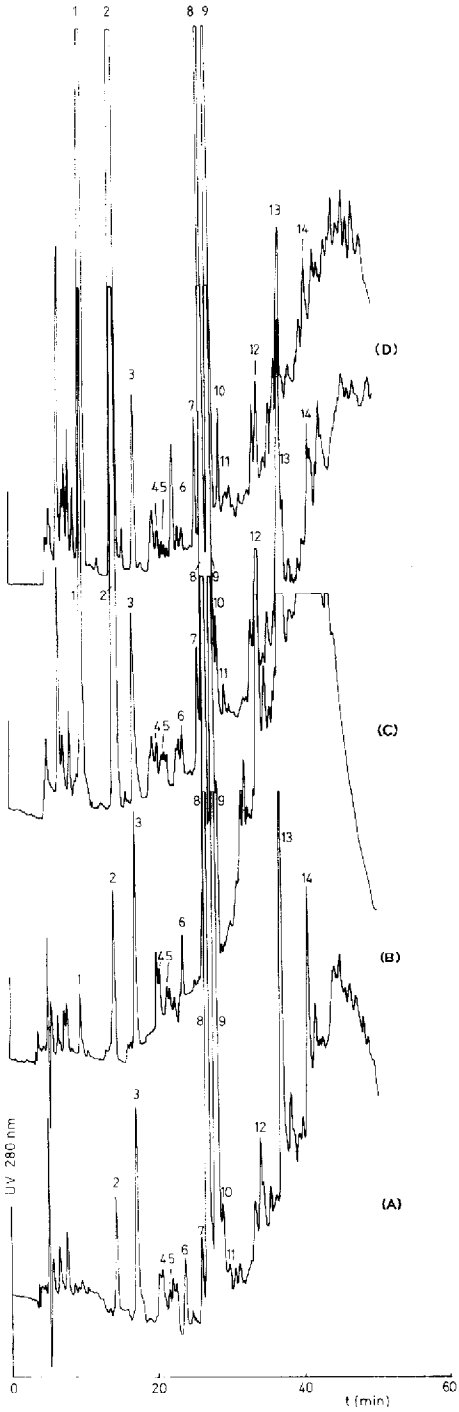

Fig. 4.

shows a number of peaks which can hardly be separated because of the large variety of bonds in dimeric and oligomeric lignin fragments.

Fig. 4 demonstrates the separation of lignin degradation products and sugar decomposition products of the main fraction as a function of the reaction temperature; the flow-rate was kept constant within the reaction vessel at 10 and 7.5 ml/min, respectively. By comparing the elution curves, it is seen that the main lignin products show relatively constant concentration values. In addition, the main fractions contain similar amounts of reaction products with almost the same product distribution. A rise in reaction temperature results in a strong increase in the amount of sugar decomposition products, *e.g.*, HMF (1) and furfural (2). All the analysed samples contained a nearly identical amount of *p*-hydroxybenzoic acid (3). At the same time the amounts of coniferyl alcohol (8) and sinapyl alcohol (9) decrease, because these substances are unstable at higher temperatures. Furthermore, the chromatograms include a peak, due to the sum of different high-molecular-weight compounds. This peak is not present when prefractionation by means of GPC has been carried out prior to analysis. From a comparison of the chromatograms in Fig. 4, it is seen that the hydrothermolysis products of the main fraction show an invariable pattern.

The course of the reaction can best be studied when the fractions are collected as a function of time at a predetermined temperature and a constant flow-rate. In the first phase of a degradation experiment at 240°C (Fig. 5), at the initial heating period, only small amounts of lignin are brought into solution. At the same time only small concentrations of HMF are formed. In contrast, after reaching the desired reaction temperature, the formation of furfural is predominant. The concentration of lignin degradation products, such as coniferyl alcohol (8), sinapyl alcohol (9), pinoresinol (14), etc., decreases with reaction time. This can be explained by the low stability of these compounds. A number of lignin degradation products, *e.g.*, *p*-hydroxybenzoic acid (3), vanillic acid (4), homovanillic acid (5), syringic acid (6), vanillin (7) and syringaldehyde (10), remain nearly constant over the whole reaction period. At the end of the reaction the predominant products are HMF (1) and furfural (2). The high concentration of the latter compound is due to the elimination of formaldehyde from HMF.

After the degradation of lignin a number of compounds of different groups such as phenols, phenolic aldehydes, ketones and organic acids may be present. In Fig. 5b a chromatogram of a reference mixture is given, showing the separation of aromatic aldehydes and ketones. However, a comparison of this chromatogram with the chromatograms of lignin degradation solutions shows that identification of the products formed throughout the reaction is not possible only by evaluation of reten-

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Fig. 4. HPLC elution curves of lignin and sugar decomposition products from hydrothermal degradation of poplar wood, as a function of the reaction temperature. (A) Reaction temperature: 180°C. Flow-rate: 10 ml/min. Solid matter of the fraction: 6.9 mg/ml. Dissolved lignin: 31% of the initial amount. (B) Reaction temperature: 200°C. Flow-rate: 10 ml/min. Solid matter of the fraction: 13.6 mg/ml. Dissolved lignin: 68% of the initial amount. (C) Reaction temperature: 240°C. Flow-rate: 10 ml/min. Solid matter of the fraction: 16.5 mg/ml. Dissolved lignin: 71% of the initial amount. (D) Reaction temperature: 280°C. Flow-rate: 7.5 ml/min. Solid matter of the fraction: 33.7 mg/ml. Dissolved lignin: 76% of the initial amount. Peaks: 1 = HMF; 2 = furfural; 3 = *p*-hydroxybenzoic acid; 4 = vanillic acid; 5 = homovanillic acid; 6 = syringic acid; 7 = vanillin; 8 = coniferyl alcohol; 9 = sinapyl alcohol; 10 = syringaldehyde; 11 = acetovanillon; 12 = 4-hydroxypropiophenone; 13 = propioguaiacon; 14 = pinoresinol.

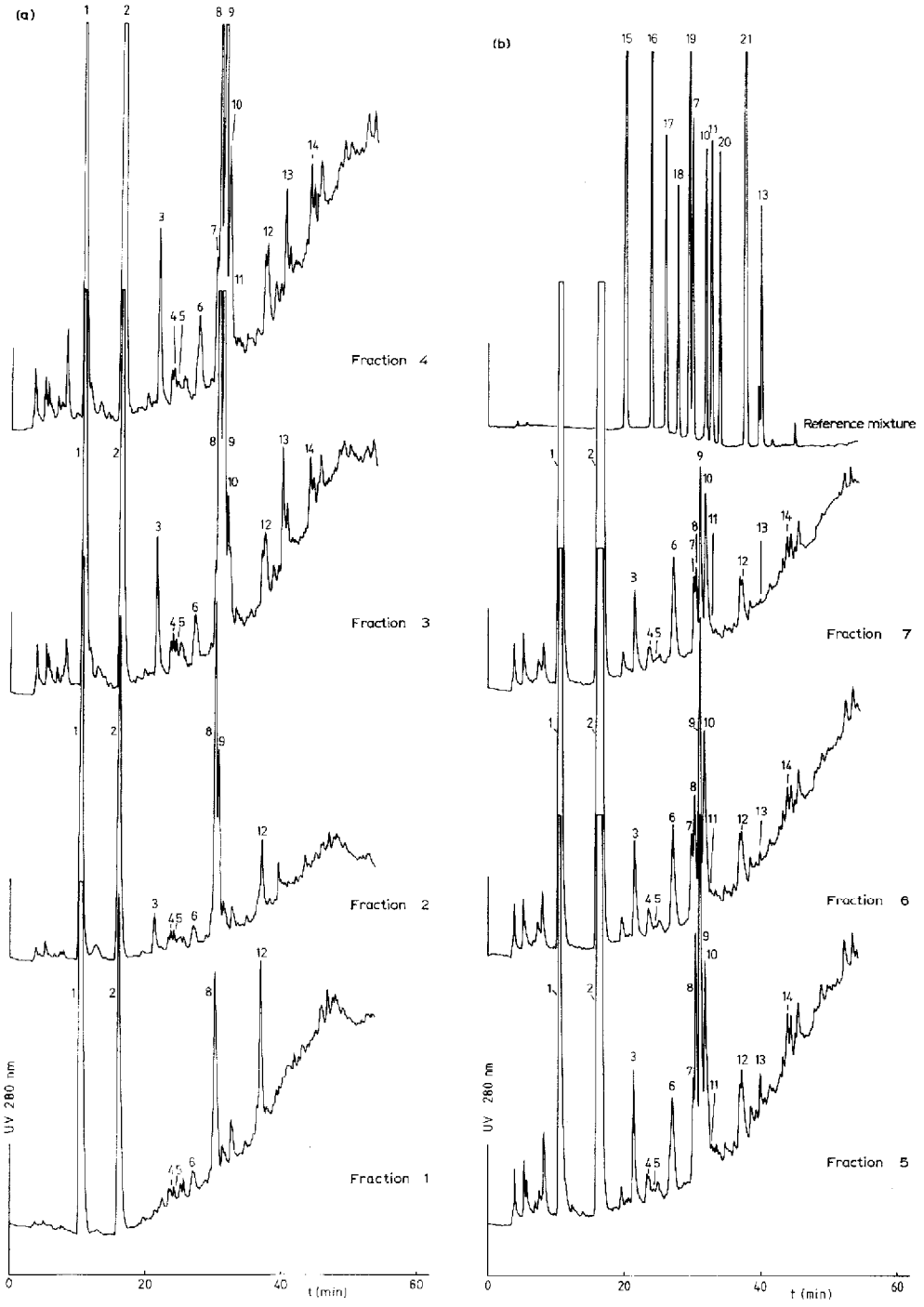

Fig. 5. (a) HPLC chromatograms of consecutive fractions from hydrothermolysis of poplar wood at 240°C with a flow-rate of 5 ml/min; decomposition products are shown as a function of reaction time. For peak identification: see Fig. 4. Solid matter in fractions; 1, 1.0 mg/ml; 2, 5.3 mg/ml; 3, 18.5 mg/ml; 4, 18.9 mg/ml. (b) Continuation of (a) including a chromatogram of reference substances (aldehydes and ketones). Peaks: see Fig. 4; 15 = 3,4-dihydroxybenzaldehyde; 16 = 3,4-dihydroxyacetophenone; 17 = 4-hydroxybenzaldehyde; 18 = α-hydroxypropiovanillon; 19 = 4-hydroxyacetophenone; 20 = acetosyringone; 21 = veratraldehyde. Solid matter in fractions: 5, 13.0 mg/ml; 6, 8.8 mg/ml; 7, 6.0 mg/ml.

tion times. Also the internal standard method allows no well defined identification. For this reason, analyses by means of GC–MS were also carried out, which enabled an exact interpretation of the chromatograms[58]. After GC separation of the resulting products and identification by means of mass spectrometry, reference substances were used as internal standards for HPLC analysis of the degradation solutions. The evaluation of the results from both methods (HPLC and GC–MS) always gave the same spectrum of substances. Therefore HPLC can be applied for investigation of the course of reaction. Moreover, the HPLC chromatograms show a negligible number of overlapping peaks, caused by small amounts of less important substances.

CONCLUSIONS

The liquid chromatographic determination of the product distribution of monomeric sugars, lignin degradation products and sugar decomposition products allows a rapid optimization of hydrothermolysis with respect to the reaction temperature, flow-rate and dimensions of the reaction vessel. If a certain main product is desired, these LC methods give valuable information about the reaction conditions which have to be applied. In addition, by means of HPLC methods, the kinetics of individual biomass components can be evaluated.

The analytical procedures described can also be employed successfully in the investigation of other biomass conversion processes, *e.g.*, hydrogenolytic degradation or oxidative hydrolysis of lignin. HPLC enables a very rapid and quantitative analysis of the main products without time-consuming sample preparation. For an exact peak identification of lignin degradation compounds, GC–MS analyses are, however, required.

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