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Size-exclusion chromatography of lignocellulosics in wheat straw

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ABSTRACT

The application of Fractogel TSK HW-40, a semi-rigid, hydrophilic vinyl polymer, and Vydac 214 TP, a wide-pore high-performance liquid chromatographic packing, to the fingerprinting of extracts of wheat straw, both untreated and treated with sodium hydroxide, is reported. Whereas separation on Fractogel TSK is typical size-exclusion chromatography, chromatography on the Vydac column is influenced by both adsorption effects and the molecular size of the solutes. Chromatograms showing significant differences among samples are discussed in relation to sample pretreatment and extraction, molecular weight distribution and electrochemical properties of the main peak.

INTRODUCTION

Lignocellulosic materials, such as wood, agricultural residues and industrial by-products, are an economical and renewable source of energy and raw material in areas of application as diverse as the chemical industry, fibre and paper manufacture, livestock feeding and even environmental decontamination from toxic chemicals. Lignocellulose resources represent about 95% of the world biomass [1]. Cellulose, hemicellulose and lignin are the three main constituents of lignocellulose. Lignin is a polymer with phenylpropane units, linked to each other by carbon–carbon and ether bonds. Lignin forms complex structures with cell-wall polysaccharides (lignin–carbohydrate complexes, LCC) through hydrogen and ether bonding [2]. Lignin depolymerization and/or disaggregation of the complexes with polysaccharides is often the basis of practical processes of lignocellulose exploitation, such as animal feeding with straw, where an improved digestibility of structural carbohydrates can be achieved by treating straw with alkali or lignin-degrading organisms.

Lignin degradation products have been successfully analysed by gas chromatography (GC) [3] and reversed-phase high-performance liquid chromatography (HPLC) [4–6]. Direct analysis of lignocellulosic substrates has been carried out by Fourier transform infrared (FTIR) spectrometry [7], ^{13}C nuclear magnetic resonance (^{13}C NMR) [8] and pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS)

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[9]. These techniques provide data on the phenolic monomers related to lignin, allowing chemical characterization and significant comparisons among treated and untreated materials, but give no information on the molecular weight distribution of native lignin, oligomers derived from lignin degradation or lignin–carbohydrate complexes.

Size-exclusion chromatography (SEC) and high-performance SEC (HPSEC) are well established methods for determining the molecular weight distribution of industrial lignins, although relatively few publications have dealt with SEC of lignin from gramineous plants and LCC [10]. Molecular weight determination is not as straightforward for lignins and LCCs as for synthetic polymers, owing to a lack of standard compounds with a chemical nature similar to that of lignin (for column calibration) and possible effects that disturb the relationship between column elution volume and polymer hydrodynamic volume, such as adsorption, aggregation and ionic/hydrophobic interactions [11]. Separation media used for the SEC of lignins and LCCs from gramineous plants include soft and semi-rigid gels such as Sephadex, Sepharose and Sephacryl types, whereas HPSEC has been performed on rigid gels after various modes of chemical deactivation, such as μStyragel (polystyrene–divinylbenzene), μBondagel E (bonded silica) and Zorbax PSM (silica) [10,11].

The aim of this work was to establish whether significant fingerprintings of wheat straw samples were obtainable by using two packing materials so far not reported for the characterization of molecular weight distributions in lignocellulose from gramineous plants. Extracts of wheat straw, both untreated and treated with sodium hydroxide to upgrade its digestibility by ruminants, were chromatographed on Fractogel TSK HW-40(S) and on Vydac 214 TP. This chromatographic support is a porous spherical gel with high chemical and mechanical stability for SEC and Vydac 214 TP is a wide-pore (300 Å) short-chain (C_4)-bonded support designed for the reversed-phase separation of peptides and other large molecules.

EXPERIMENTAL

Sample preparation

A 200-mg amount of each of an untreated sample (control, C) and a sample treated with sodium hydroxide (3% of dry matter), ground to pass a 0.2-mm sieve, was extracted with 10 ml of buffer [26.3 m*M* EDTA disodium salt dihydrate–58.2 m*M* KH_2PO_4–40.6 m*M* $Na_2HPO_4 \cdot 2H_2O$ (pH 7)] for 1 h under reflux. After cooling and centrifugation, the supernatant was decanted, filtered through a 0.22-μm cartridge filter (Millipore), diluted to 25 ml with the eluent and injected into the liquid chromatograph. The residue was extracted with 10 ml of 0.1 *M* sodium hydroxide solution for 10 min at 120°C and subjected to the same procedure as above, prior to injection into the liquid chromatograph.

Chromatographic conditions

Condition 1. Fractogel TSK HW-40(S) (swollen particle size 25–40 μm) (Merck, Darmstadt, F.R.G.) was packed into a Merck Superformance glass column (300 × 10 mm I.D.) and connected to a liquid chromatographic system, consisting of a Model 590 pump (Waters Assoc., Millford, MA, U.S.A.), a Rheodyne Model 7010 sample injector (100-μl loop), a Waters Assoc. Model 440 UV detector (set at 280 nm)

and a Model 5011 dual-cell detector (ESA, Bedford, MA, U.S.A.) controlled by an ESA Coulochem 5100 A module. Chromatograms were displayed on a Model 561 recorder (Perkin-Elmer, Beaconsfield, U.K.). The mobile phase was 1.2 m*M* perchloric acid at 1 ml/min. Analyses were performed at room temperature.

Condition 2. A Vydac 214 TP 54 column (300 × 4.6 mm I.D.) was connected to a Perkin-Elmer LC 4 liquid chromatograph with a Rheodyne 7125 S injector (6-μl loop), equipped with a Perkin-Elmer LC 95 variable-wavelength UV–VIS detector set at 280 nm. The mobile phase was water–acetonitrile (9:1) containing 0.5% trifluoroacetic acid at a flow-rate of 0.8 ml/min.

RESULTS AND DISCUSSION

Fractogel TSK is a semi-rigid gel, consisting of hydrophilic vinyl polymers with numerous hydroxyl groups on the matrix surface. Fractogel TSK is therefore completely different, in both its chemical structure and microstructure, from the usual gels based on dextran, agarose and polyacrylamide [12].

The molecular weight (MW) operating range of Fractogel TSK HW-40 is from 100 to 8000 for dextrans and from 100 to 2000 for polyethylene glycols. Oligomers detected in studies on lignin degradation were of such an MW order [11]. It therefore seemed appropriate to start this study on SEC of lignocellulose with this medium.

Considering that SEC of lignocellulose suffers from a lack of proper calibration compounds, the column was calibrated under condition 1 (see Experimental) with dextrans and sugars (Sigma, St. Louis, MO, U.S.A.) using a refractive index detector. Calibration results were as follows: blue dextran, void volume (V_0) 6.6 min; dextran with average MW 9000, 7.00 min; dextran tetramer–decamer mixture with MW from *ca.* 660 to *ca.* 1630, 9.9 min; dextran with MW 500, 12.4 min; sucrose with MW 342, 13.3 min; and glucose with MW 180, 14.5 min.

An acidic eluent was used in previous work [4–7] because it prevents adsorption effects of phenolic substances on reversed-phase packings. In this work, acidic eluents were used for both size-exclusion and reversed-phase chromatography in order to make a homogeneous comparison of the two packings.

Fig. 1 shows the four chromatograms corresponding to the sample preparations described under Experimental. The comparison between pH 7 buffer extracts of the control and sodium hydroxide-treated wheat straw (Fig. 1a and b) showed a lower total response in the control than in the treated sample, owing to the presence in the latter of more extractables, derived from sodium hydroxide hydrolysis of cell-wall polymers. There were also differences in the relative distributions of the molecular weights of the minor compounds (peaks A, C and D), corresponding approximately to the MW at the size-exclusion limit, 900 and 400 (dextran calibration). The main peak, B, corresponds to an MW of *ca.* 2300 (dextran calibration). In addition to the absorption at 280 nm, peak B had an electrochemical activity that proved its phenolic nature. The study of the voltammetric behaviour of an HPLC peak is generally performed by recording its hydrodynamic voltammogram (HV). The HV of peak B, determined by repeated injections at different detector potentials, showed a minimum detectable oxidation response at +0.45 V and a maximum at +1.00 V, with an intermediate plateau between +0.60 and +0.70 V, which suggests the presence of hydroxyl substituents in *ortho* and *meta* positions.

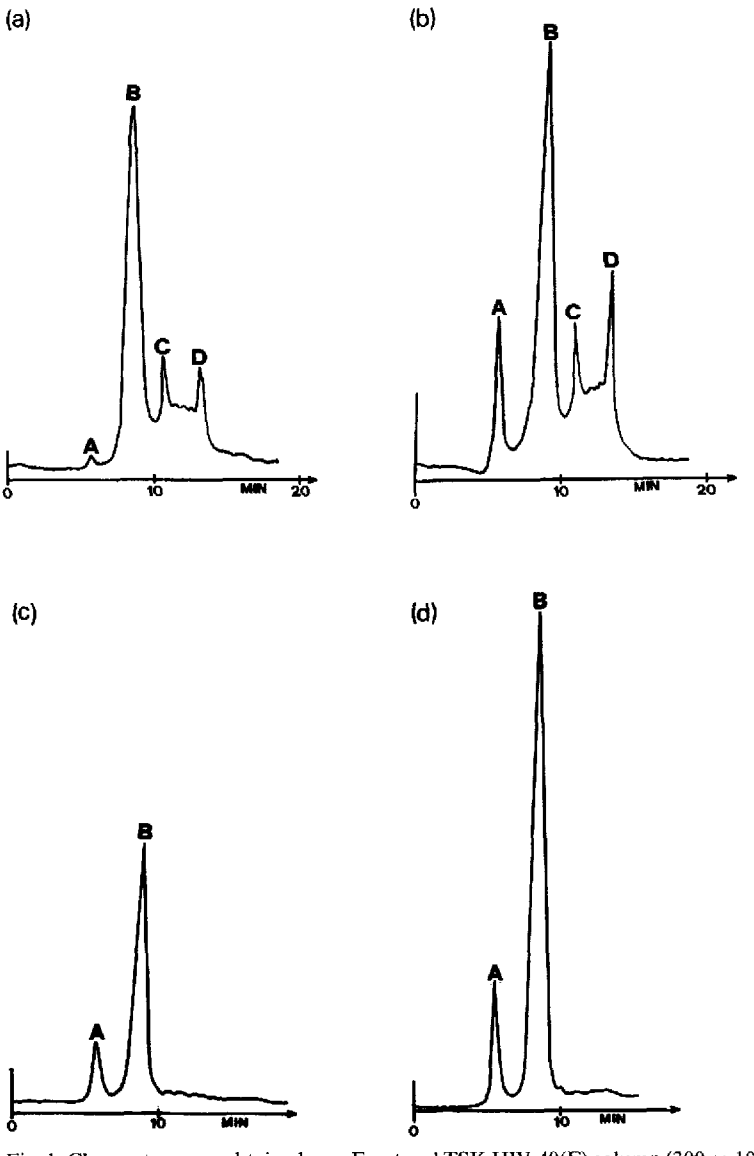

Fig. 1. Chromatograms obtained on a Fractogel TSK HW-40(F) column (300 × 10 mm I.D.) with detection at 280 nm of pH 7 buffer extracts of (a) control and (b) sodium hydroxide-treated wheat straw, and of the sodium hydroxide extracts of the corresponding residues (c) and (d). The sensitivity of (a) and (b) is double that of (c) and (d). Eluent, 1.2 m*M* perchloric acid; flow-rate, 1 ml/min.

In the sodium hydroxide extracts (Fig. 1c and d) there is a marked simplification of the chromatograms and an increased response in comparison with the previous two. Only peaks A and B are present. As observed with the other extracts, the treated sample showed a higher total response than the control. The lower molecular weight components (peaks C and D) are not present in the sodium hydroxide extracts,

probably because they are compounds loosely bound to the cell-wall polymers and, therefore, easily removed by the pH 7 buffer.

Wide-pore (300-Å) packings can be used for the reversed-phase separation of high-molecular-weight biological substances, such as proteins [13]. As no application of such columns to the characterization of the MW distribution in lignocellulose samples has been reported, it seemed interesting to perform a chromatographic separation of the same extracts on a column of this type.

Fig. 2 shows the elution profiles of the same extracts as in Fig. 1, obtained on a Vydac 214 TP C_4 column. The two columns show a consistent behaviour in terms of relative enhancement of response when sodium hydroxide-treated straw and sodium hydroxide extracts are compared with control straw and pH 7 buffer extracts, respectively. However, chromatographic separations on such columns can be affected by both adsorption effects and the molecular size of the solute. It is therefore more difficult to attribute chromatographic peaks to a certain molecular weight distribution in the sample, especially with lignocellulose owing to the already cited lack of calibration standards of appropriate structure. Nevertheless, given the differences in the fingerprinting of different straw samples, such a column is worthy of further study.

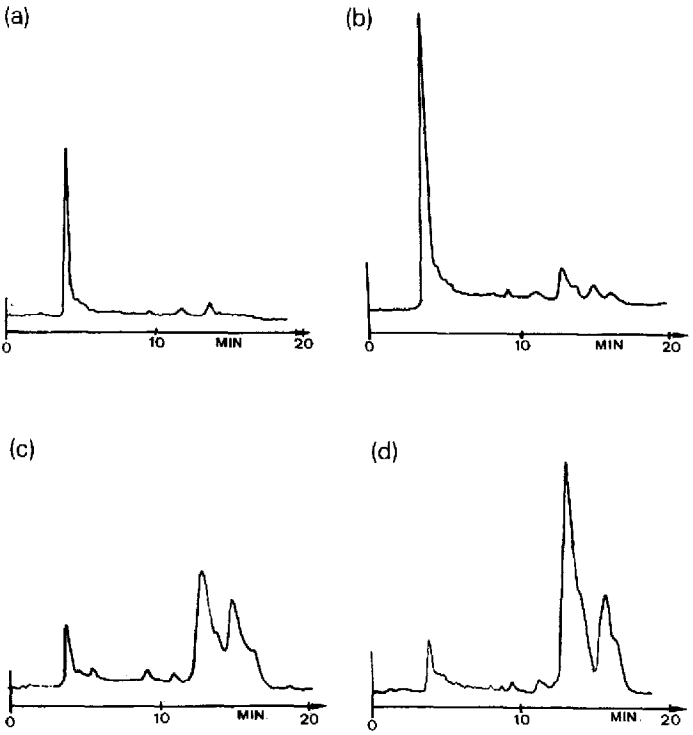

Fig. 2. Same extracts as in Fig. 1, chromatographed on a Vydac 214 TP column (300 × 4.6 mm I.D.) with detection at 280 nm. Eluent, water–acetonitrile (9:1) containing 0.5% trifluoroacetic acid; flow-rate, 0.8 ml/min.

CONCLUSION

Molecular weight determinations are tenuous on the basis of conventional chromatographic standards and, therefore, further analyses of the collected fractions should be performed in order to obtain absolute data. However, a fingerprinting comparison is satisfactory when only relative MW distributions or changes due to sample treatments have to be determined. The analysed samples, although small in number, allow the conclusion that TSK HW-40 is a useful gel for the SEC of lignocellulosic samples. Separations are rapid in comparison with the classical gels used for low-pressure SEC, and a rough MW distribution of these samples is obtainable. Vydac 214 TP chromatograms are interesting but more difficult to interpret. This kind of approach is now common in the study of LCCs and should be investigated further for application to lignocellulosic samples.

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