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# High-performance size-exclusion chromatography of wood hemicelluloses on a poly(2-hydroxyethyl methacrylate–co-ethylene dimethacrylate) column with sodium hydroxide solution as eluent

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ABSTRACT

The determination of the molecular mass parameters of wood hemicelluloses by high-performance size-exclusion chromatography (HPSEC) using a poly(2-hydroxyethyl methacrylate–co-ethylene dimethacrylate) (Separon S HEMA 1000) column and 0.5 *M* NaOH as solvent and eluent is described. It is shown that universal calibration between dextran and xylan is valid. The Mark–Houwink equation for xylan in 0.5 *M* NaOH was found to be $[\eta] = 2.67 \cdot 10^{-4} M^{0.73}$, where $M$ = molecular mass. A direct method of characterization of hemicelluloses extracted with 18% NaOH from different celluloses and pulps by HPSEC is described.

## INTRODUCTION

Hemicelluloses are a type of polysaccharide that is widespread together with cellulose in land plants. Large amounts are present in wood tissues, and some of them remain in the wood pulp, therefore influencing the pulp properties.

The degree of polymerization (*DP*) and polydispersity of wood hemicelluloses have been investigated by numerous workers over many years [1–3]. The molecular mass ($M_r$) parameters of wood hemicelluloses have been studied by viscometry [4–6], osmometry [1,4–7] and ultracentrifugation [1,4]. Hemicelluloses are established to have a *DP* of 300–30, and polydispersity values are quoted from monodisperse to highly polydisperse [1–6].

Size-exclusion chromatography has been the most popular method for determining the molecular mass distribution (MMD) of hemicelluloses, traditionally carried out using soft organic gels as column packing materials [8–12]. Today for the determination of the MMD of polymers the time-consuming gel permeation chromatography has generally been replaced by high-performance size-exclusion chromatography (HPSEC). However, the determination of the MMD of hemicelluloses utilising HPSEC columns is still very limited.

In a previous paper [13], the feasibility of determining the MMD of the 4-O-methyl-D-glucuronoxylan in aprotic solvents (dimethyl sulphoxide, dimethylformamide (DMF) by HPSEC, using a poly(2-hydroxyethyl methacrylate–co-ethylene dimethacrylate) (Separon S HEMA 1000) column, was investigated. The results showed that fractionation according to MM was achieved, using DMF containing appropriate amounts of $H_3PO_4$ and LiBr as eluent. Further experiments demonstrated that the Separon S HEMA 1000 packing is a stable in

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strongly alkaline media and it is possible to determine the MMD of hemicelluloses using cadoxen (pH ≈ 13) as eluent [14,15]. A disadvantage of this method is that large amounts of cadoxen, an expensive and laborious way to prepare eluent, are needed.

In this work, in order to develop a more convenient and rapid method for the determination of the MMD of hemicelluloses by HPSEC, the use of a prepacked Separon S HEMA 1000 column with 0.5 *M* NaOH as eluent was investigated.

## EXPERIMENTAL

### *Chemicals and materials*

Analytical reagent-grade sodium hydroxide (Lachema, Brno, Czech Republic) and doubly distilled water were used. Dextran standards T 10, T 20, T 40, T 70, T 110, T 500 and T 2000 were obtained from Pharmacia (Uppsala, Sweden). Oat husk xylans were obtained from Sigma (St. Louis, MO, USA) and Serva (Heidelberg, Germany).

### *Isolation of the hemicelluloses*

The preparation and characterization of the xylan fractions were described elsewhere [13]. The isolation procedures for birch and beech xylans and arabinogalactan were described previously [13,16,17]. Spruce glucomannan was isolated from spruce chlorite holocellulose by the usual procedure, as described for the glucomannan [3].

### *Extraction of the hemicelluloses fractions*

A 400-mg amount of milled pulp fibres or holocellulose was placed in a 10-ml tube, 4 ml of 18% NaOH were added and the mixture was left for 1 h at room temperature. The sample was quickly squeezed and filtered through an N2 glass filter and then immediately analysed by HPSEC.

### *Solution preparation*

The solvent for all samples was 0.5 *M* NaOH, except for larch arabinogalactan, for which water was used as the solvent. The time for complete dissolution was 10–20 min. To calibrate the chromatographic system and to analyse isolated hemicelluloses samples, 25 $\mu$l of 0.2% solutions were injected. The hemicelluloses were found to be stable in 0.5 *M* NaOH at least for 6 h; the chromatograms after this time were virtually identical with these obtained immediately after the dissolution.

### *HPSEC*

The analyses were performed on a size-exclusion chromatograph from Laboratory Instruments (Prague, Czech Republic) with a refractometric detector, equipped with a Rheodyne Model 7125 fixed-loop (100 $\mu$l) injector. A prepacked stainless-steel column (250 × 8 mm I.D.) containing Separon S HEMA 1000 (10 $\mu$m) (Tessek, Prague, Czech Republic) was used. The eluent was 0.5 *M* NaOH and analyses were carried out at the room temperature. The analysis time was 20 min at a flow-rate 0.4 ml/min.

### *Intrinsic viscosity measurement*

Viscosities were determined at 25 ± 0.05°C in cadoxen using an Ubbelohde viscometer. The $DP_v$ of the hemicelluloses samples was obtained from viscometric measurements using the following equation, established for xylan [4]; $[\eta] = 9.2 \cdot 10^{-3}\ DP_v^{0.84}$ dl/g.

## RESULTS AND DISCUSSION

### *Calibration of column*

A series of dextrans were used to obtain the calibration graph and to check the range of retention volume for chromatographic separation. The $M_r$ parameters of dextran standards provided by the manufacturer are given in Table I.

Fig. 1 shows typical elution curves for dextran standards under the conditions applied. The fractionation of dextrans T 10, T 20, T 40, T 70 and T 110 is resulted in symmetrical curves; dextrans T 500 and T 2000 were only partially resolved since a portion of them have $M_r$ values higher than the column exclusion limit. A plot of 0.5 log ($\bar{M}_w\bar{M}_n$) against retention volume ($V_e$) gives a straight line over the range $3 \cdot 10^5$–$2 \cdot 10^3$ (Fig. 2).

TABLE I

MOLECULAR MASS AND ELUTION CHARACTERISTICS OF DEXTRAN STANDARDS AND XYLAN FRACTIONS

$\bar{M}_w$ = mass-average molecular mass; $\bar{M}_n$ = number-average molecular mass; $M_p$ = peak molecular mass calculated by 0.5 ln $(\bar{M}_w\bar{M}_n)$.

| No. | Sample | $\bar{M}_w$ | $\bar{M}_n$ | $M_p$ | $V_e$ (ml) | Log $M_p[\eta]$ |
|---|---|---|---|---|---|---|
| 1 | Dextran T 2000 | 2 000 000 | | | 3.96 | 6.21 |
| 2 | Dextran T 500 | 506 000 | 190 000 | 310 000 | 4.08 | 5.24 |
| 3 | Dextran T 110 | 116 000 | 56 000 | 81 700 | 4.87 | 4.38 |
| 4 | Dextran T 70 | 66 300 | 36 400 | 49 100 | 5.25 | 4.05 |
| 5 | Dextran T 40 | 39 400 | 23 400 | 30 400 | 5.58 | 3.75 |
| 6 | Dextran T 20 | 20 900 | 14 000 | 17 100 | 6.00 | 3.38 |
| 7 | Dextran T 10 | 9 500 | 4 900 | 6 800 | 6.58 | 2.78 |
| 8 | Xylan I | 26 200 | 20 800 | 23 300 | 5.33 | 3.98 |
| 9 | Xylan II | 22 300 | 17 700 | 19 900 | 5.47 | 3.87 |
| 10 | Xylan III | 17 600 | 13 100 | 15 200 | 5.70 | 3.66 |
| 11 | Xylan IV | 13 000 | 9 600 | 11 170 | 5.94 | 3.43 |
| 12 | Xylan V | 11 000 | 7 900 | 9 300 | 6.09 | 3.29 |
| 13 | Xylan VI | 5 800 | 4 200 | 4 900 | 6.62 | 2.81 |
| 14 | Xylan 0 | 19 000 | 14 000 | 16 300 | 5.64 | 3.72 |
| 15 | Raffinose | 504 | | | 7.25 | |
| 16 | Cellobiose | 342 | | | 7.40 | |
| 17 | Glucose | 180 | | | 7.52 | |

The xylan fractions were used to obtain a calibration graph for evaluation the MMD of hemicelluloses. The $M_r$ and retention volumes of the fractions are given in Table I. As can be seen from Fig. 2, the calibration graph for xylans is linear over the range examined.

The investigation of the elution behaviour of hemicelluloses showed that there are no non-exclusion effects observed on the Separon S HEMA 1000 column in 0.5 *M* NaOH.

The possibility of universal calibration applied to the calculation of the MMD of hemicelluloses was examined. For this purpose, the Mark–Houwink constants for xylan fractions in 0.5 *M* NaOH were calculated using combined SEC and viscometric data according to the principle described by Dobbin *et al.* [18]. The Mark–Houwink equation was $[\eta] = 2.67 \cdot 10^{-4} M^{0.73}$ dl/g, where $M$ = molecular mass. It has been found that when using for dextran the constants $K = 13.2 \cdot 10^{-2}$ and $\alpha = 0.478$ [19], and the evaluated constants for xylan, the universal calibration {log

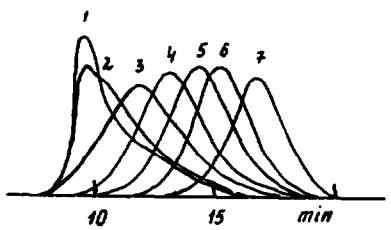


Fig. 1. Experimental chromatograms of dextran standards (numbers as in Table I).

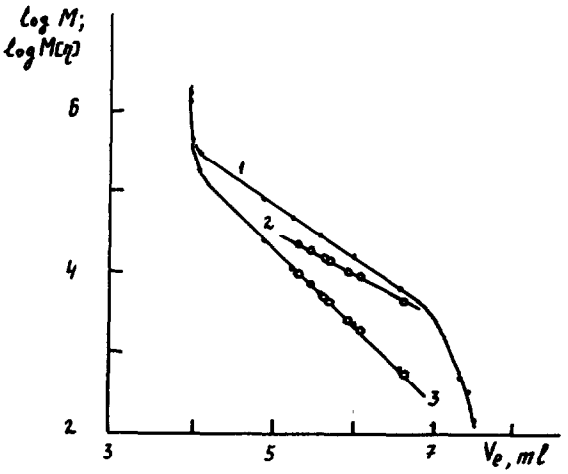


Fig. 2. Calibration graphs of log $M = f(V_e)$ for (1) dextran and (2) xylan and (3) universal calibration of log $M[\eta] = f(V_e)$ obtained on the Separon S HEMA 1000 column with 0.5 *M* NaOH.

$M[\eta] = f(V_e)\}$ is valid, as shown in Fig. 2. Hence the universal calibration procedure can be used to calculate the $M_r$ parameters of hemicelluloses if characterized hemicellulose fractions are unavailable.

*Analysis of isolated hemicelluloses*

In order to demonstrate the ability of the column to fractionate hemicelluloses according to $M_r$, the following hemicelluloses samples were used: two wood xylans isolated from birch and beech holocelluloses, two oat husk xylans supplied by Sigma and Serva, glucomannan isolated from spruce holocellulose, arabinogalactan from larch and two hemicellulose fractions from spruce sulphite and pine kraft viscose-grade bleached celluloses isolated at the mercerization stage.

As Fig. 3 shows, the chromatograms of the investigated hemicelluloses are nearly symmetrical. The $M_r$ parameters of these samples calculated by calibration of xylan fractions are given in Table II. The xylans from oat husks have a higher $M_r$ than those isolated from hardwood holocelluloses. Thus, the mass-average molecular mass $\bar{M}_w$ of the Sigma and Serva xylans are *ca.* 25 000, whereas those of beech and birch xylans are *ca.* 20 000 and 17 000, respectively. The pine kraft hemicelluloses differ from those obtained from spruce sulphite cellulose. They have a higher $M_r$ values and are less polydisperse: $\bar{M}_w = 8900$, $M_w/M_n = 1.30$ and $\bar{M}_w = 5900$, $M_w/M_n = 1.42$, respectively.

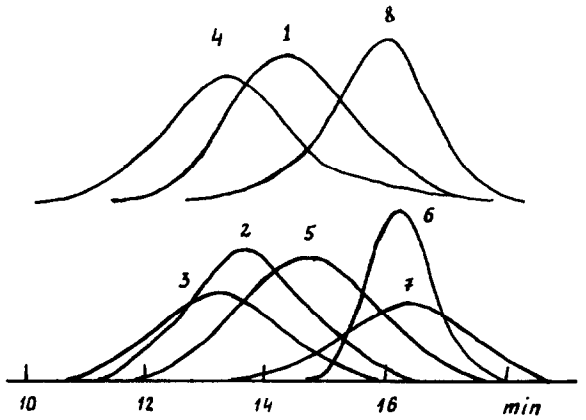


Fig. 3. Experimental chromatograms of isolated hemicelluloses (numbers as in Table II).

The $M_r$ data for reference xylans obtained by SEC are in good agreement with $\bar{M}_v$ (viscosity-average molecular mass) estimated by viscometry in cadoxen and are closely comparable to the results obtained by the other investigators for the corresponding hemicelluloses [1–3]. The data obtained show that the calibration graph evaluated for xylans is applicable to the $M_r$ calculation of softwood glucomannan, giving reliable results. Thus, the $DP_w$ values of softwood glucomannan have been reported to be from 60 to 90 and $\bar{M}_w$ from 10 000 to 15 000, respectively [1–3]; these results are in good agreement with ours (Table II).

TABLE II

MOLECULAR MASS PARAMETERS OF ISOLATED HEMICELLULOSES

| No. | Sample | $\bar{M}_w$ | $\bar{M}_n$ | $M_w/M_n$ | Viscometry in cadoxen | |
|---|---|---|---|---|---|---|
| | | | | | $\bar{M}_v$ | $DP_v$ |
| 1 | Xylan, birch | 17 510 | 12 600 | 1.39 | 17 000 | 109 |
| 2 | Xylan, beech | 20 650 | 14 440 | 1.43 | 20 000 | 133 |
| 3 | Xylan, oat husks (Sigma) | 27 500 | 20 500 | 1.34 | 26 100 | 174 |
| 4 | Xylan, oat husks (Serva) | 26 270 | 17 170 | 1.53 | 25 000 | 166 |
| 5 | Glucomannan, spruce | 14 600 | 11 000 | 1.45 | 14 000 | 86 |
| 6 | Arabinogalactan, larch | 6 600 | 5 500 | 1.21 | – | – |
| 7 | Hemicelluloses from spruce sulphite cellulose | 5 900 | 4 150 | 1.42 | 5 350 | 33 |
| 8 | Hemicelluloses from pine sulphate cellulose | 8 900 | 6 830 | 1.30 | 8 500 | 53 |

For the larch arabinogalactan $M_r$ data, it should be noted that no reliable data have been reported previously and the estimated earlier $M_r$ values seems to be too high [11,20]. It is our suggestion that these values for arabinogalactan $M_r$ are high owing to the polyelectrolyte expansion and the $M_r$ of arabinogalactan in fact does not exceed 10 000 [16].

*Analysis of alkaline extracts*

The species of hemicelluloses are traditionally obtained by extraction with basic solutions; commonly 10–24% KOH or NaOH are used, followed by precipitation and purification [1–3]. In this work, the analysis of alkaline extracts from wood pulps was performed without isolation of the hemicelluloses from the solution; no sample treatment other than extraction and filtration was required. This procedure shortens the analysis time considerably and minimizes any changes in the hemicelluloses fractions due to sample preparation.

Fig. 4 shows the SEC of the hemicelluloses extracted with 18% NaOH from birch holocellulose, spruce sulphite paper- and viscose-grade pulps and beech and pine kraft viscose-grade pulps. The volume injected for extracts from holocellulose and paper-grade samples was 25 $\mu$l and for viscose-grade samples 100 $\mu$l.

The $M_r$ parameters of the extracted hemicelluloses are given in Table III. These data are compared with the corresponding values for hemicelluloses presented in Table II. The average $M_r$ and polydispersity of extracted birch xylan and isolated by the usual procedure from holocellulose are virtually identical. The hemicelluloses extracted from pine kraft and spruce sulphite viscose-grade bleached celluloses have higher average $M_r$ values than the isolated compounds. Obviously the extracted hemicelluloses suffer less extensive changes than those isolated at the mercerization stage.

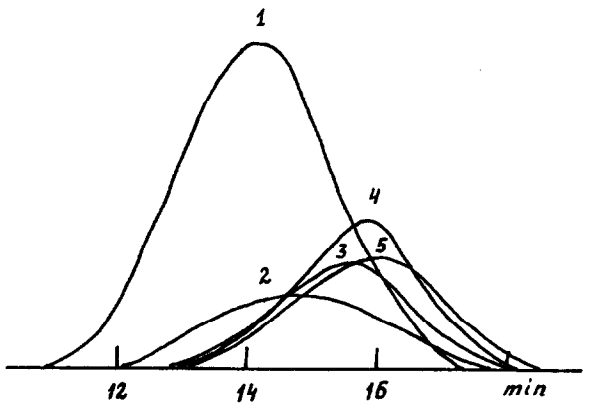


Fig. 4. Experimental chromatograms of wood hemicelluloses extracted with 18% NaOH from (1) birch holocellulose, (2) beech sulphate viscose-grade, (3) spruce sulphite paper-grade, (4) pine sulphate and (5) spruce sulphite viscose-grade celluloses.

It should be pointed out that simultaneously in addition to the determination of the MMD of the hemicelluloses, it is also possible by HPSEC to determine the hemicellulose content in the extracts obtained from different wood celluloses.

TABLE III

MOLECULAR MASS PARAMETERS AND AMOUNTS OF HEMICELLULOSES EXTRACTED WITH 18% NaOH FROM CELLULOSES

| No. | Cellulose sample | Solubility in 18% NaOH (%) | | $\bar{M}_w$ | $\bar{M}_n$ | $M_w/M_n$ |
|---|---|---|---|---|---|---|
| | | Standard method | HPSEC | | | |
| 1 | Birch holocellulose | 33.0[a] | 30.0 | 17 470 | 12 640 | 1.38 |
| 2 | Spruce sulphite paper-grade cellulose | 13.0 | 13.5 | 8 240 | 5 500 | 1.50 |
| 3 | Beech sulphate viscose-grade cellulose | 3.9 | 4.2 | 10 120 | 6 910 | 1.46 |
| 4 | Spruce sulphite viscose-grade cellulose | 5.0 | 5.1 | 9 100 | 6 250 | 1.46 |
| 5 | Pine sulphate viscose-grade cellulose | 4.2 | 4.3 | 9 410 | 6 580 | 1.43 |

[a] Pentosan content determined according to ref. 3.

For this purpose calibration graphs for the corresponding hemicelluloses with known concentrations *vs.* chromatographic peak area obtained by refractive index detection were constructed. The calculated amounts of hemicelluloses in the investigated wood celluloses are summarized in Table III. The data obtained by HPSEC are in good agreement with the hemicellulose content determined by standard analytical methods [3].

Further investigations in this direction are in progress, and more detailed information on the HPSEC analysis of alkali-soluble fractions from different wood celluloses will be published.

## CONCLUSIONS

An HPSEC method for the determination of the MMD of hemicelluloses, both isolated by a commonly used procedure and directly extracted with 18% NaOH, has been described. The method suggested for the characterization of wood hemicelluloses is very simple, because stages such as neutralization, precipitation and purification, which are usually required in hemicellulose isolation procedures, are omitted. All changes in and losses of hemicelluloses connected with the traditional scheme of isolation in this instance are eliminated.

Further, the HPSEC method can be used to determine the hemicellulose content in different wood celluloses and pulps when the corresponding calibration graph has been obtained. The method presented is useful for measurements of numerous samples in routine work.

The Separon S HEMA 1000 column is particularly suitable for the determination of the MMD of different hemicelluloses. The possibility of using this SEC technique for studies of any alkali-soluble polymer is evident.

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