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PREPARATION OF XYLODEXTRINS AND THEIR SEPARATION BY GEL CHROMATOGRAPHY

W. BROWN AND Ö. ANDERSSON
Institute of Physical Chemistry, Uppsala University, Uppsala (Sweden)*

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SUMMARY

The preparation of the straight chain xylodextrins, xylose-xyloheptaose, from Esparto grass xylan is described. The nature of the separation on polyacrylamide and dextran gels has been investigated and comparisons are made with the analogous cellodextrins. Good correlation is found between the separations on dextran gels and in thin-layer chromatography on Kieselguhr. It is concluded that solubility-determined partitioning is an important factor in separations on tightly cross-linked Dextran gels.

INTRODUCTION

This contribution describes the preparation of the straight chain xylodextrins that were required for use as model substances in current investigations on the surface properties of cellulosic networks employing chromatographic techniques. Xylan, a hemicellulose, is a low-molecular-weight polysaccharide (degree of polymerization, approx. 200) of variable structure occurring in plant cell walls together with cellulose and in woody materials also with lignin. The xylan in Esparto grass, for example, is a straight-chain polymer of β-(1-4), linked D-xylopyranose units[1]. In hardwoods, the xylan contains one-unit branches of the 4-O-methyl ether of D-glucuronic acid on the main xylose backbone. In other xylans (*e.g.* barley husks and maize) there are L-arabinose units in non-terminal positions.

EXPERIMENTAL

Hydrolysis of xylan

The hydrolysis conditions employed were essentially those described by WHISTLER AND TU[2] for xylan from corncob holocellulose.

Esparto grass xylan** (20 g) was added in small portions under stirring to 1500 ml of fuming HCl at −15°. The latter was prepared by passing HCl gas through conc. HCl cooled in a mixture of ice and NaCl (2:1). After 30 min the dissolution of xylan

* Address: Box 532, 751 21 Uppsala 1, Sweden.

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was complete (contrasting with the immediate dissolution of cellulose in this medium). The reaction mixture was allowed to warm to 0° and was maintained at this temperature for 40 min. The hydrolysis was stopped by adding the mixture to 2.4 kg of $NaHCO_3$ in 2 kg of ice. A few milliliters of *n*-octanol were added to reduce frothing when the mixture was stirred. The final pH was 6.0. The liquid was filtered after standing overnight and applied directly to the active charcoal column.

Fractionation of xylan hydrolysate

The fractionation procedure was a modification of that described by WHISTLER AND DURSO[3].

Darco G-60 active charcoal (Darco Corp., N.Y., 2 kg) and the same quantity of A. R. grade Celite was suspended by vigorous stirring for 1½ h in a 2.5% solution of stearic acid in 16 l of abs. ethanol. The stearic acid partially de-activates the charcoal and facilitates sequential elution of the oligosaccharides. The mixture was filtered and the adsorbent re-suspended in 16 l of stearic acid-saturated 50% aq. ethanol. Following filtration, the adsorbent was re-suspended in 10 l of 10% aq. ethanol and vigorously stirred for 2½ h to effect good dispersion of charcoal and Celite. The slurry was poured into a 113 cm^2 × 100 cm plexiglass column and the settled bed (80 cm) washed with 20 l of distilled water.

Following introduction of the hydrolysate, the bed was washed with 40 l of distilled water to remove salts and xylose. The oligosaccharides were then obtained by gradient elution using 20 l of de-ionized water in one container connected through a siphon to an equal volume of 50% aq. ethanol in a second container. A hydrostatic pressure of 2.5 m was applied giving a flow rate of 15 ml/min. Aliquots (0.1 ml) of every 10th fraction were analyzed for carbohydrate concentration using the orcinol reagent (3 ml of 0.2% orcinol in 70% H_2SO_4). After boiling for 20 min, the absorbance of the cooled solution was measured at 550 nm.

The fractions were also analyzed by thin-layer chromatography (TLC) as described below. The fractions were pooled according to the TLC results and each pool was concentrated to about 200 ml in a rotary evaporator at 45°. The residues were freeze-dried to yield the quantitites of xylodextrins listed in Table I.

Thin-layer chromatography

Plates of Kieselguhr G (20 × 20 cm) buffered with 0.02 *M* sodium acetate were employed. The solvent system aq. isopropanol (65%)–ethyl acetate (1:1) was used

TABLE I

YIELDS OF XYLODEXTRINS FROM CHARCOAL–CELITE CHROMATOGRAPHY

Xylodextrin	*Yield (g)*
Xylobiose	1.15
Xylotriose	1.04
Xylotetraose	0.55
Xylopentaose	0.29
Xylohexaose	0.12
Xyloheptaose	1.28

TABLE II

VALUES OF THE MIGRATION PARAMETER, R_F, IN TLC (KIESELGUHR G; 65% AQ. ISOPROPANOL–ETHYL ACETATE, 1:1)

Xylodextrins	R_F	*Cellodextrins*	R_F
Xylose	0.93_8	Glucose	0.87_4
Xylobiose	0.56_7	Cellobiose	0.79_2
Xylotriose	0.24_1	Cellotriose	0.60_4
Xylotetraose	0.11_4	Cellotetraose	0.36_3
Xylopentaose	0.05_2	Cellopentaose	0.21_6
Xylohexaose	—	Cellohexaose	0.10_3

for development. The cellodextrin series, previously described[4], was also run in parallel with the xylodextrin series. Values of the migration parameter, R_F, are given in Table II.

Molecular weight determination

It was necessary to confirm the identification of the first fraction as xylobiose since the behavior of the xylodextrins and cellodextrins is very different in the TLC system used. The molecular weight was measured* using a Hewlett-Packard, Model 302, vapor pressure instrument. The latter was calibrated with glucose and operated at 39° with water as solvent. The ordinate intercept of the plot in Fig. 1 gives a value $M_n = 300$, in good agreement with the theoretical value for xylobiose (282). The melting point also agreed with the figure (185°) reported by WHISTLER AND TU[2].

Gel chromatography

The preparation of the polyacrylamide P-2 and Sephadex G-15 columns was as

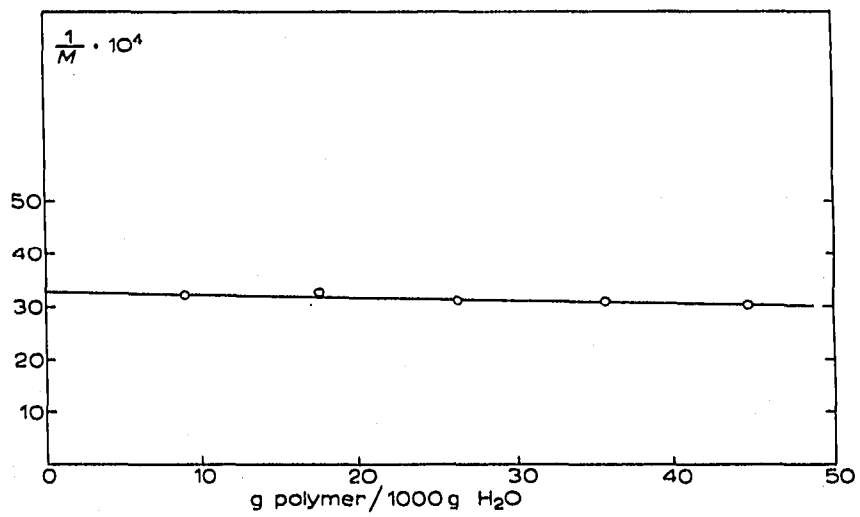

Fig. 1. Vapor pressure data for xylobiose fraction.

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TABLE III

DIMENSIONS OF GEL CHROMATOGRAPHY COLUMNS

	Polyacrylamide P-2	*Sephadex G-15*
Settled bed volume	47.7 ml	45.5 ml
Void volume, V_0 (Blue dextran)	17.1 ml	19.0 ml
Internal volume, V_i	28.7 ml	17.0 ml
Column height	60 cm	58 cm
Cross-sectional area	0.785 cm^2	0.785 cm^2
Flow rate	2 ml/h	3 ml/h
Sample volume	0.1 ml	0.1 ml
Sample concentration	1 mg/ml	1 mg/ml

previously described[4]. Ref. 4 also gives details of the detection system employed. The dimensions of the columns are summarized in Table III.

RESULTS AND DISCUSSION

As expected with the random degradation of polysaccharide chains on acid hydrolysis[5], the yields (Table I) of the lower members of the xylodextrin series are the highest, with the noteworthy exception of xyloheptaose. Although it has long been accepted that xylan from Esparto grass is composed of linear chains of uniform composition of D-xylopyranose units[1], the high yield of the heptaose (and its presence as a contaminant in the other fractions) would point to some form of discontinuity at every seventh unit at which initial chain scission occurs. There is apparently some evidence for the pressure of a single branch point in Esparto xylan. There are not, however, side branches constituting glucuronic acid residues as in the hardwood xylans[6], as it was established that the fractions do not contain ionizable groups.

Polyacrylamide gel chromatography

Elution volumes and the corresponding partition coefficients on polyacrylamide

TABLE IV

GEL CHROMATOGRAPHY DATA FOR XYLODEXTRINS (POLYACRYLAMIDE P-2; WATER)

Xylodextrin	V_e *(ml)*	K_D	$-\Delta \log K_D$
Xylose	40.5_4	0.93_4	
			0.076
Xylobiose	38.8_3	0.86_0	
			0.077
Xylotriose	37.2_2	0.80_2	
			0.087
Xylotetraose	35.5_2	0.73_5	
			0.085
Xylopentaose	34.0_0	0.67_5	
			0.068
Xylohexaose	33.4_6	0.63_1	
			0.016
Xyloheptaose	32.64	0.62_1	

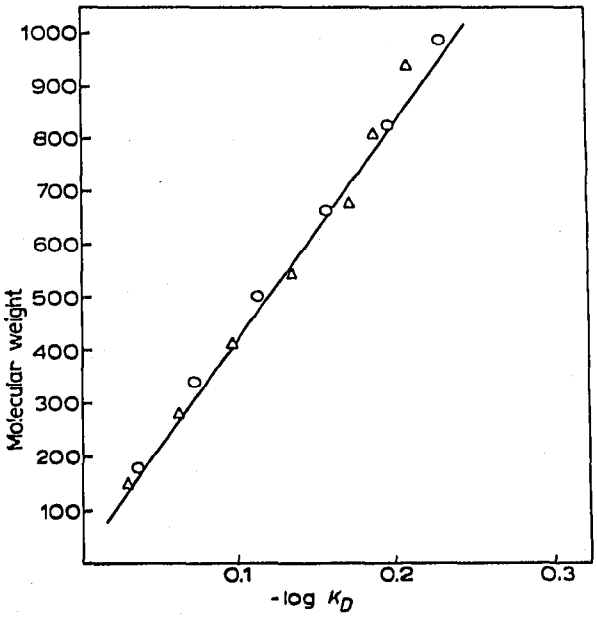

Fig. 2. Gel chromatography relationship between molecular weight and $-\log K_D$ for xylodextrins and cellodextrins (Polyacrylamide P-2, deionized water).

P-2 are listed in Table IV. Fig. 2 shows a plot of $-\log K_D$ *versus* molecular weight with the data for the cellodextrins[4] included for comparison. The data for the xylodextrins and cellodextrins can be approximated by a common line, indicating that the molecular size is the dominant factor governing the separation in this system. This contrasts with the separations on Sephadex in which the solubility of the solute has a strong influence (see below). Adsorption of the oligosaccharides to the polyacrylamide gel also occurs, as previously noted[4].

Dextran gel chromatography

Elution volumes and partition coefficients for the xylodextrins and cellodextrins on Sephadex G-15 are listed in Table V. Fig. 3 shows a plot of $-\log K_D$ *versus* molecular weight.

The partition coefficient for a given xylodextrin is significantly smaller than

TABLE V

GEL CHROMATOGRAPHY DATA FOR XYLODEXTRINS AND CELLODEXTRINS (SEPHADEX G-15, 0.1 *M* NaCl)

	Xylodextrins			*Cellodextrins*	
	V_e	K_D		V_e	K_D
Xylose	34.1_0	0.88_8	Glucose	33.0_0	0.82_4
Xylobiose	28.1_0	0.53_6	Cellobiose	30.3_0	0.66_5
Xylotriose	25.6_0	0.38_8	Cellotriose	27.9_0	0.52_4
Xylotetraose	24.2_5	0.30_0	Cellotetraose	26.2_0	0.42_3
Xylopentaose	23.2_0	0.24_7	Cellopentaose	24.7_0	0.33_6
Xylohexaose	22.5_5	0.20_0	Cellohexaose	24.0_0	0.29_4
Xyloheptaose	22.2_0	0.18_8	Celloheptaose	23.2_0	0.24_7

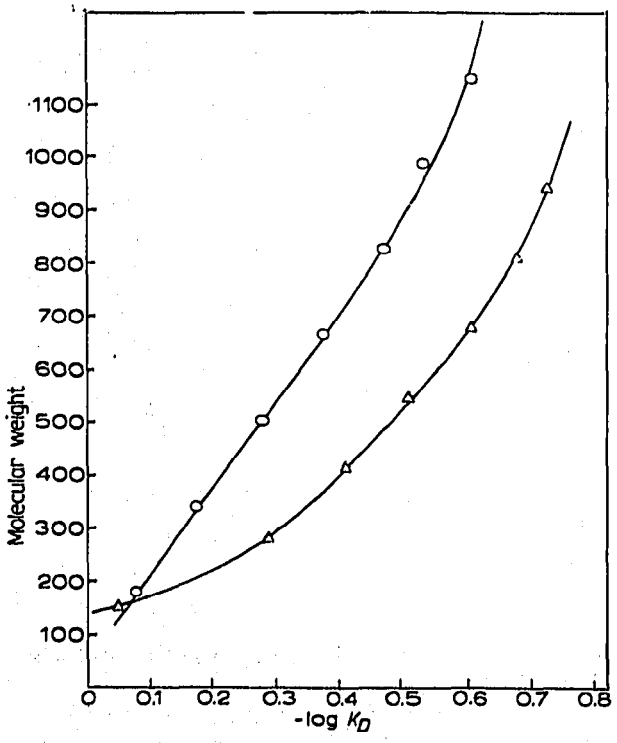

Fig. 3. Gel chromatography: relationship between molecular weight and $-\log K_D$ for xylodextrins and cellodextrins (Sephadex G-15, 0.1 *M* NaCl).

that for the analogous cellodextrin (with the exception of the monomers). This shows that the cellodextrins are strongly retarded, an effect which becomes more pronounced with increasing molecular weight.

It becomes clear that the underlying processes governing the separations on polyacrylamide and dextran gels differ considerably and are more complex in the latter. On the one hand volume exclusion causes the larger molecular species to elute first, a process which is common to both gel types. As solute molecules pass through the gel in the mobile phase they are assumed to be in equilibrium with those in the stationary phase. Since solute molecules of different size "see" different volumes in the latter, they will elute at different volumes. With Sephadex, however, there also appears to be a solubility-determined liquid–liquid partitioning of the solute between the stationary and mobile solvent phases (or between bulk solvent and bound solvent). Thus the observed partition coefficient will be the product of $K_{\text{volume exclusion}}$ and $K_{\text{solubility partition}}$.

This is demonstrated by the close correlation with liquid–liquid chromatography on an inert support as described below. On the dextran gel, solutes of lower solubility (less hydrophilic) favour the stationary water phase. This is illustrated by MARSDEN's data[7] for the separation of the alkane diols on Sephadex G-25—the least hydrophilic of the two solutes of similar molecular size exhibits the larger partition coefficient.

The partitioning effect determined by solubility thus functions in opposition to volume exclusion and the two processes tend to counterbalance each other at the higher molecular weights (see Fig. 3 of ref. 4). The influence of the opposing effects is

demonstrated by the change in the trend of the partition coefficients for the N-alcohols on Sephadex[7] where partitioning becomes more important than volume exclusion with increasing chain length.

This mechanism fits the present data for separations on the dextran gel. Although the xylodextrins and corresponding cellodextrins are similar in molecular size (and thus the molecular weight–log K_D relationships on polyacrylamide coincide; see Fig. 2), they have differing solubilities. Since glucose is more hydrophilic than xylose it has a lower K_D value. With increasing chain length the cellodextrins become less hydrophilic than the xylodextrins owing to intramolecular hydrogen bonding involving the primary hydroxyls at C_5. The oligomeric cellodextrins consequently exhibit greater K_D values.

Thin-layer chromatography

The importance of solubility partitioning on tightly cross-linked dextran gels is shown by the similarity of the separations to those in thin-layer chromatography.

Values of the migration parameter, R_F, are given in Table II for the xylodextrins and cellodextrins. The function $(1-R_F)/R_F$ is simply related to the liquid–liquid partition coefficient, K, by

$$\left(\frac{1-R_F}{R_F}\right) \cong K \cdot \frac{\emptyset_s}{\emptyset_m}$$

where $\emptyset_s$ and $\emptyset_m$ are the volume fractions of the stationary and mobile solvent phases. A plot of $\log[(1-R_F)/R_F]$ *vs.* molecular weight is shown in Fig. 4; it bears a close resemblance to Fig. 3. The more hydrophilic xylodextrins migrate more slowly than the cellodextrins due to their greater affinity for the stationary aqueous phase. This does not apply to the monomers since, as noted above, glucose is more hydrophilic than xylose.

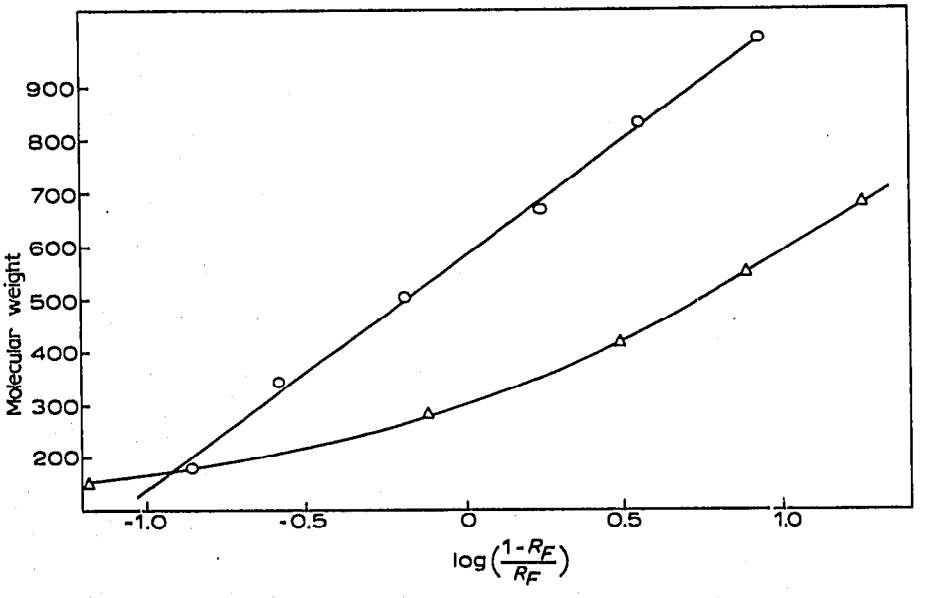

Fig. 4. TLC data: relationship between molecular weight and log $(1-R_F)/R_F$ for xylodextrins and cellodextrins (Kieselguhr G; 65% aq. isopropanol–ethyl acetate, 1:1).

Kieselguhr is considered to be quite inert and apparently holds the aqueous phase in the form of dispersed micro-drops[8]. Consequently, the thin-layer system should approximate true liquid–liquid chromatography and the separations reflect the relative hydrophilicities of the solutes. ISHERWOOD AND JERMYN[9] have shown that with the separations of oligosaccharides, $\log[(1-R_F)/R_F]$ is a linear function of $\log N$ where N is the mole fraction of water in the mobile phase.

The TLC and dextran gel chromatography data are combined in Fig. 5 showing $\log[(1-R_F)/R_F]$ as a function of $-\log K_D$. A single line adequately represents the data for both oligosaccharide series and shows the close correlation between the

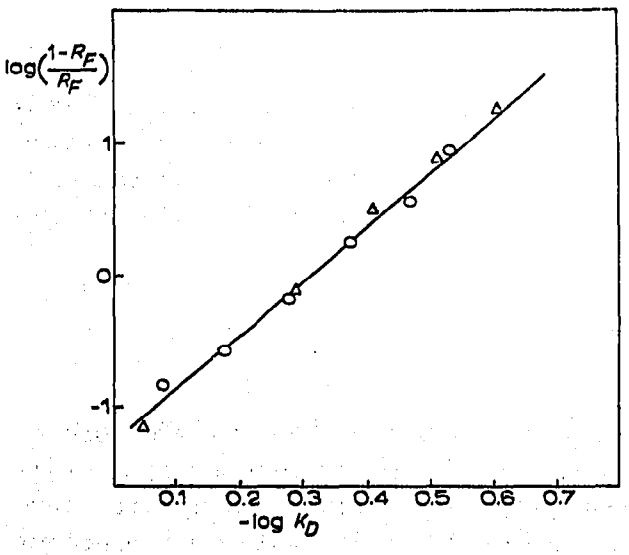

Fig. 5. Relationship between TLC data and gel chromatography data (Sephadex G-15) for xylodextrins and cellodextrins.

separations in TLC and dextran gel chromatography. The affinity of the less hydrophilic solutes for the stationary solvent phase could be a result of adsorption. The present results only show that the stationary phase is favoured by relatively hydrophobic solutes. There is good evidence that the cellodextrins bind strongly to cellulose gels[10]. It would be surprising, however, if the differing adsorptivities of the xylodextrins and cellodextrins are so well described by their partitioning behaviour in the TLC system.

The conclusion that liquid–liquid partitioning based on solubility behaviour is an important factor in separations on dextran gels is not restricted to sugar separations but is apparently generally valid. One may expect, however, that volume exclusion will be dominant in separations of high molecular weight polymers on more open networks.

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