CHROM. 5853

Separation of xylodextrins by gel chromatography

A previous communication[1] described the preparation and separation of the straight chain xylodextrins (β-(1-4) linked D-xylopyranose units). Subsequent analyses have shown that the materials isolated and referred to as xylobiose (X2) up to and including xyloheptaose (X7) were incorrectly identified as such and are X4 up to X9, respectively. While the qualitative conclusions drawn from the data in ref. 1 are unchanged, this note presents the corrected tabulated data and figures redrawn to accord with them. Similarly, the data for X2 to X6 in ref. 2, refer to X4 to X8.

Discussion

Polyacrylamide gel. Table I lists partition coefficients, K_{av}, for the xylodextrins on Polyacrylamide P-2 with 0.1 M NaCl and deionized water as eluants. Corresponding data for the cellodextrins[3] are included. K_{av} is used here to avoid the ambiguity involved in determinations of the internal solvent volume of the gel, V_I, particularly when gel-solute interactions are known to occur.

$$K_{av} = \frac{(V_e - V_o)}{(V_T - V_o)}; \qquad K_D = \frac{(V_e - V_o)}{V_I}$$

V_e, V_o and V_T are the solute elution volume, void volume and total volume of the gel mass in the column.

TABLE I

GEL CHROMATOGRAPHY DATA FOR XYLODEXTRINS AND CELLODEXTRINS ON POLYACRYLAMIDE P-2 (25°)

Xylodextrin	K_{av}[a] (*deionized water*)	K_{av}[b] (*0.1 M NaCl*)	*Cellodextrins*	K_{av}[c] (*deionized water*)	K_{av}[d] (*0.1 M NaCl*)
X1	0.87_4	0.81_4	G1	0.86_8	0.78_8
X4	0.71_1	0.58_5	G2	0.79_5	0.68_8
X5	0.65_8	0.51_8	G3	0.72_0	0.59_3
X6	0.59_3	0.47_0	G4	0.65_5	0.52_2
X7	0.55_4	0.42_0	G5	0.60_2	0.46_6
X8	0.53_5	0.37_0	G6	0.55_2	0.42_1

[a] V_T = 47.7 ml and V_0 = 17.0 ml.
[b] V_T = 47.2 ml and V_0 = 18.8 ml.
[c] V_T = 47.7 ml and V_0 = 17.1 ml.
[d] V_T = 46.5 ml and V_0 = 19.5 ml.

Fig. 1 shows that the xylodextrins and cellodextrins fall on the same line when $-\log K_{av}$ is plotted as a function of the partial molar volume, $\bar{V}$. The common relationship with the molar volume might be interpreted to mean that a simple exclusion mechanism is the case here. However, the low-molecular-weight polyethylene oxides elute considerably earlier revealing the existence of pronounced solute-gel interactions. The importance of the latter is demonstrated by the finding[4] that with

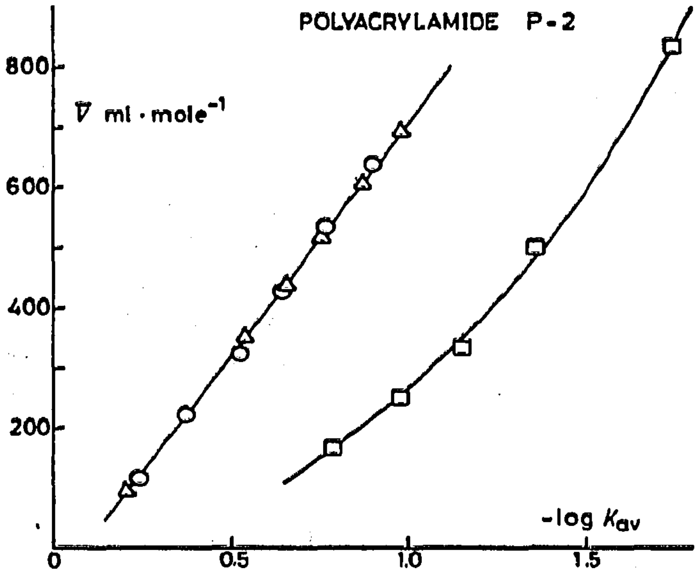

Fig. 1. Polyacrylamide P-2; 0.1 *M* NaCl. Relationships between $-\log K_{av}$ and partial molar volume for cellodextrins (○), xylodextrins (△) and polyethylene oxides (□).

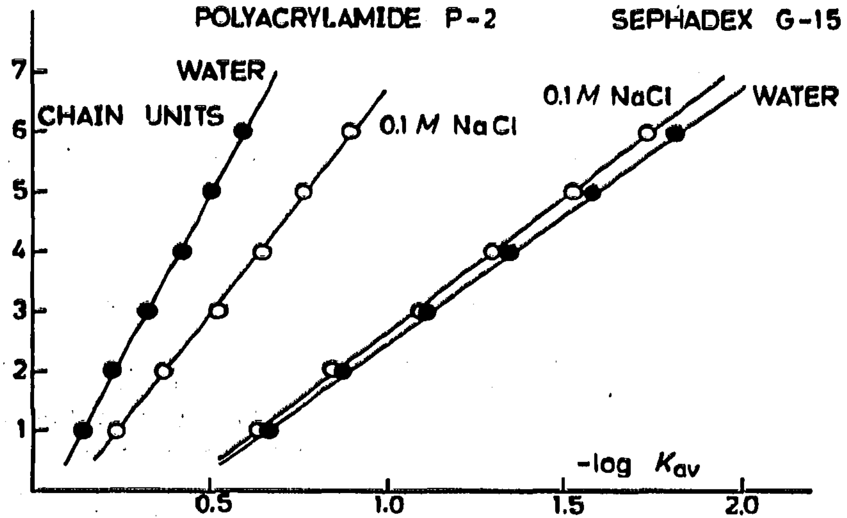

Fig. 2. Polyacrylamide and dextran gel chromatography of cellodextrins in deionized water and 0.1 *M* NaCl.

TABLE II

PARTITION COEFFICIENTS FOR XYLODEXTRINS AND CELLODEXTRINS ON SEPHADEX G-15 (25°)

Xylodextrin	K_{av}[a] (*0.1 M NaCl*)	*Cellodextrin*	K_{av}[a] (*0.1 M NaCl*)
X1	0.57_0	G1	0.52_8
X4	0.29_7	G2	0.42_7
X5	0.24_0	G3	0.33_6
X6	0.19_8	G4	0.27_3
X7	0.15_0	G5	0.21_5
X8	0.13_4	G6	0.18_0

[a] $V_T = 45.5$ ml and $V_0 = 19.0$ ml.

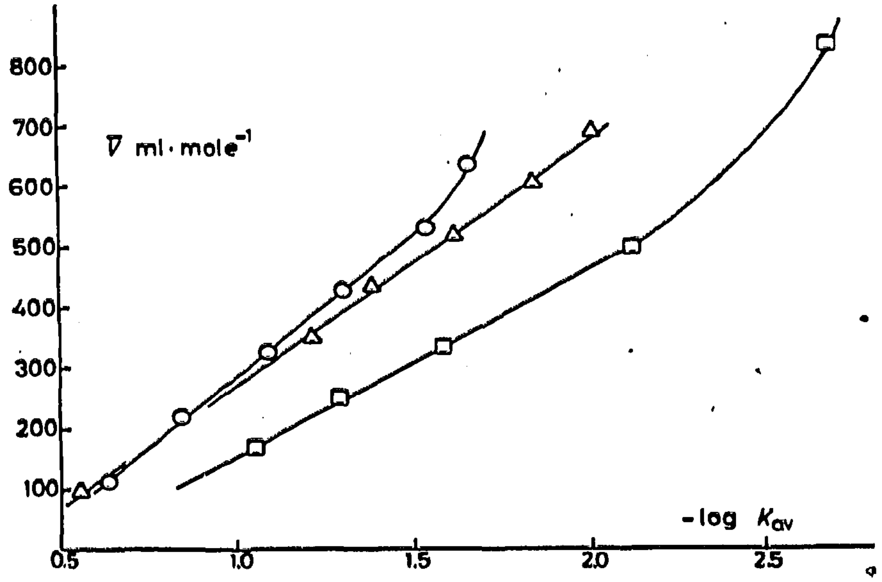

Fig. 3. Sephadex G-15; 0.1 M NaCl. Relationships between $-\log K_{av}$ and partial molar volume for cellodextrins (○), xylodextrins (△) and polyethylene oxides (□).

TABLE III

VALUES OF THE MIGRATION PARAMETER, R_F, IN TLC ON KIESELGUHR F_{254} (MERCK)

Solvent system: 42% isopropanol, 36% ethyl acetate, 23% water.

Xylodextrins	R_F	*Cellodextrins*	R_F
X1	0.75_6	G1	0.63_6
X4	0.29_2	G2	0.48_0
X5	0.15_8	G3	0.33_6
X6	0.08_7	G4	0.20_4
X7	0.04_4	G5	0.12_0
X8	0.02_2	G6	0.06_7

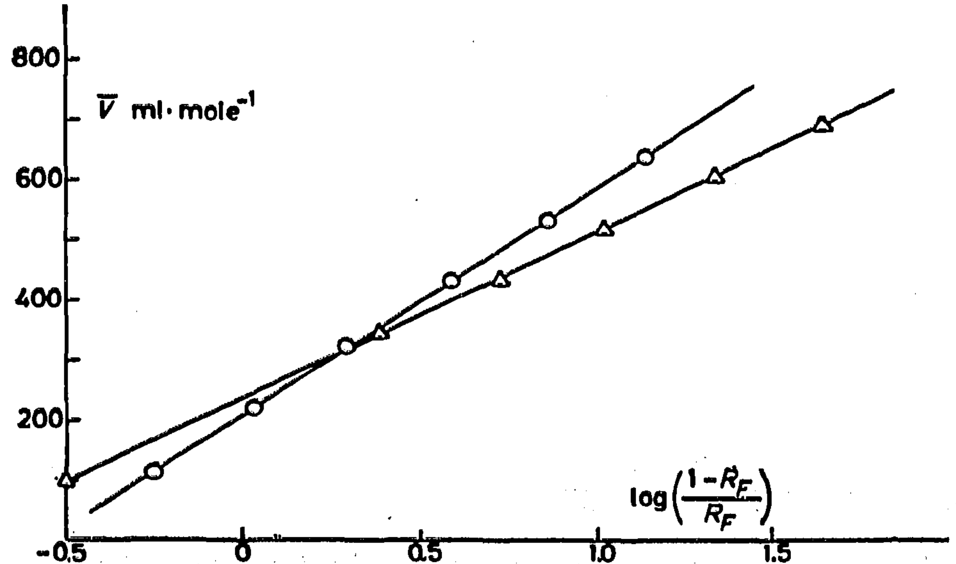

Fig. 4. TLC data as a function of partial molar volume for cellodextrins (○) and xylodextrins (△).

a hydrophobic gel (polystyrene) the polyethylene oxides elute considerably later than the oligosaccharides. The presence of salt in the eluant results in the earlier elution of the oligosaccharides, presumably due to the reduced activity of the amide groups as adsorption sites. The partition coefficients in dextran gels are relatively insensitive to the presence of salt (Fig. 2).

Dextran gel. Partition coefficients for the xylodextrins and cellodextrins on Sephadex G-15 with 0.1 M NaCl as eluant are given in Table II. Fig. 3 shows that the oligosaccharides are selectively partitioned on this gel. As in the case of the polyacrylamide gel, the earlier elution of the polyethylene oxides shows that the oligosaccharides are retarded, although to a smaller extent. The retention becomes more pronounced with increasing molecular weight.

Thin-layer chromatography (TLC) data, given in Table III, are plotted *versus* partial molar volume in Fig. 4. The function $(1-R_F)/R_F$ is simply related to the liquid–liquid partition coefficient, K, by:

$$\left(\frac{1-R_F}{R_F}\right) = K\cdot\frac{\phi_s}{\phi_m}$$

where ϕ_s and ϕ_m are the volume fractions of the stationary and mobile solvent phases. Thus (on a molar volume basis) the relatively hydrophilic xylodextrins elute first in gel chromatography. The oligomeric cellodextrins are presumably more hydrophobic than the xylodextrins owing to intramolecular hydrogen bonding between contiguous monomer units (C_6–C'_2). As expected, the order for the monomers is reversed. It is relevant that the mannodextrins[5] are more hydrophilic than the xylodextrins, probably due to the unfavourable axial orientation of the C_2 hydroxyl for such bonding. The TLC and gel chromatography data are combined in Fig. 5. The inverse proportionality

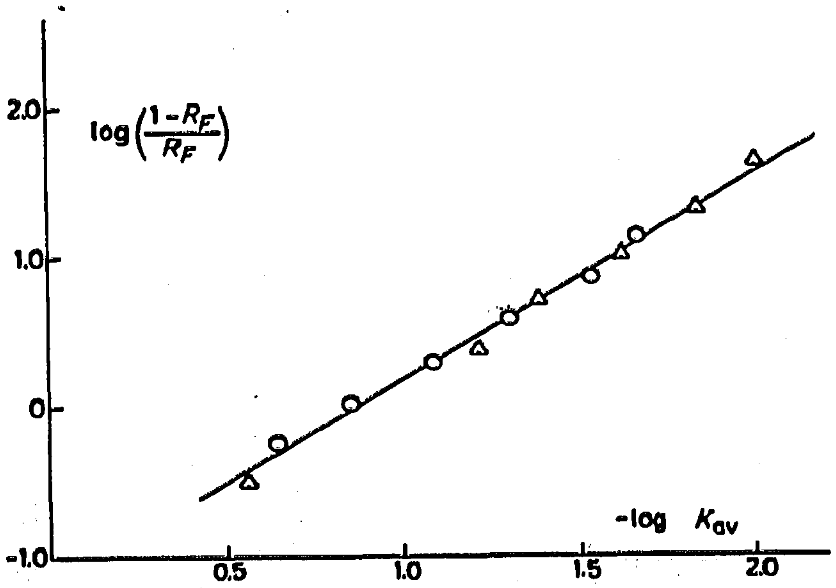

Fig. 5. Relationship between TLC data and dextran gel chromatography for cellodextrins (○) and xylodextrins (△).

shows that a common parameter (solubility behaviour) determines the separations in these very different systems.

The pronounced affinities of the oligosaccharides for the gel interface could be partly the result of physical adsorption effects. Such an interpretation fits with the temperature dependence of the partition coefficient — see below. Furthermore, the cellodextrins have been shown to chemisorb to cellulose gels[6]. The sorption tendency should then be simply related to the solubility behaviour following **Freundlich**'s expression of Traube's rule[7]: "the adsorption of organic substances from aqueous solutions increases strongly and regularly as we ascend the homologous series". It may be noted that the elution profiles are always narrow and symmetrical indicating linear partition isotherms. This is generally true with adsorption chromatography at sufficiently low concentrations.

It would thus appear that the separations of the oligosaccharides on a dextran gel are related in part to the relative polarities of the solutes and in part to their

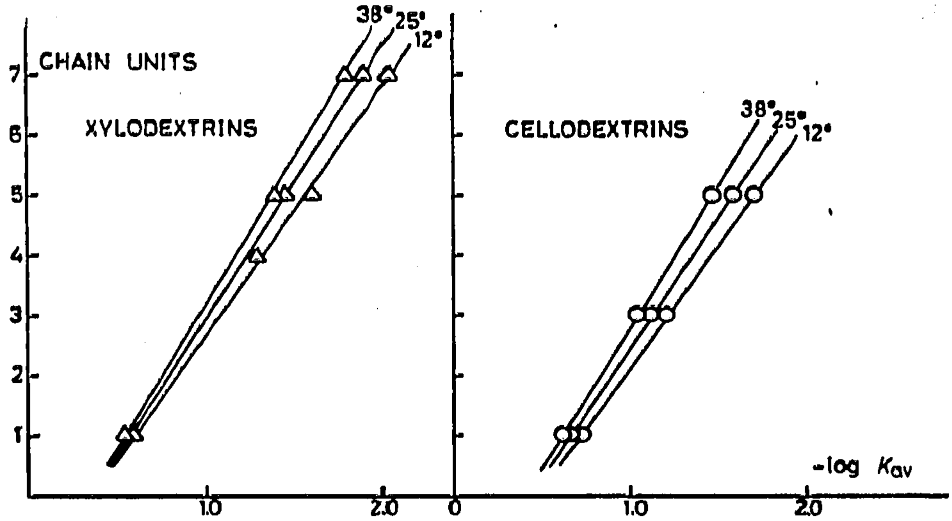

Fig. 6. The temperature dependence of K_{av} for cellodextrins (○) and xylodextrins (△) in dextran gel chromatography.

TABLE IV

ENTHALPY, FREE ENERGY AND ENTROPY PARAMETERS FOR OLIGOSACCHARIDES ON SEPHADEX G-15 AT 25°

Cellodextrin	K_{av} (25°)	$\Delta H^{0\,a}$ (*cal·mole*$^{-1}$)	$\Delta G^{0\,b}$ (*cal·mole*$^{-1}$)	$T\Delta S^{0\,c}$ (*cal·mole*$^{-1}$)
G1	0.51_3	560	395	175
G3	0.32_8	1090	660	430
G5	0.20_5	1450	940	510
Xylodextrin				
X1	0.56_8	265	335	−70
X5	0.23_8	1440	860	580
X7	0.15_1	1660	1120	540

[a] $\Delta H^0 = RT^2 \cdot \mathrm{d}\ln K_{av}/\mathrm{d}T$.
[b] $\Delta G^0 = -RT \ln K_{av}$.
[c] $T\Delta S^0 = \Delta H^0 - \Delta G^0$.

ability to couple with the surface hydroxyls. The non-specific separation on polyacrylamide and the large K_{av} values point to stronger adsorption effects with this gel, particularly with water as the eluant.

Temperature dependence of K_{av}. Fig. 6 shows that K_{av} increases with increasing temperature for both oligosaccharide series on Sephadex G-15. Values of ΔH^0, ΔG^0 and $T\Delta S^0$ are given in Table IV. The ΔH^0 values are large and positive. Basically, the phenomenon may be understood as a competition between solute and solvent molecules for occupancy of the interface. With a polysaccharide matrix and assuming a tendency for the solute to interact with the gel, *both* the decreasing gel–solvent interactions and the decreasing solubility of the polar solute with increasing temperature combine to shift the equilibrium more in favour of the gel interface. If these two factors are opposed, as is the case with the polyacrylamide gel (polyacrylamide–water interactions increase with increasing temperature[8]), the net temperature dependence of K_{av} will be small. In fact the ΔH^0-values for the cellodextrins[3] and maltodextrins[9] have been found to be negative (and small) on Polyacrylamide P-2. These results indicate a fundamental difference in the packing of water molecules in the vicinity of dextran and polyacrylamide chains. The former are heavily solvated and it is this deactivating layer that limits the interactions of the solute with the gel.

Since the partition coefficient, K_{av}, may be written:

$$K_{av} = e^{\Delta S^\circ/Re - \Delta H^\circ/RT}$$

where ΔH^0 is the net enthalpy parameter, one expects a linear relationship between —log K_{av} and ΔH^0. This is apparently the case (Fig. 7) taking into consideration the inherent difficulty in making precise measurements of the small changes in K_{av} in the small temperature range employed.

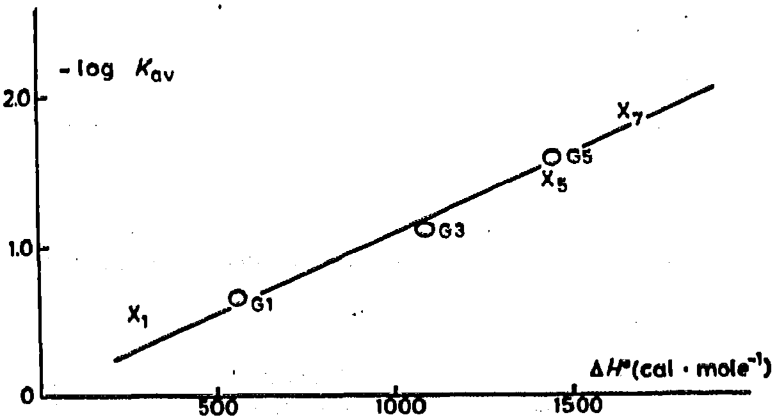

Fig. 7. Net enthalpies of adsorption, ΔH^0, as a function of —log K_{av}; cellodextrins (○) and xylodextrins (△).

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Institute of Physical Chemistry, University of Uppsala, W. BROWN
Box 532, 751 21 Uppsala 1 (Sweden) Ö. ANDERSSON

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