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THE INFLUENCE OF SOLVENT AND TEMPERATURE IN DEXTRAN GEL CHROMATOGRAPHY

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SUMMARY

The influence of solvent and temperature on the separations of oligosaccharides on dextran gels has been studied. Increasing temperature leads to an increasing partition coefficient, reflecting a greater activity of the solute in the gel phase when there are weaker gel–solvent interactions. Similarly, it was found that the better the solvent (the stronger the solvent–gel matrix interactions), the more the partition coefficient is changed to favour the mobile solvent phase. These results demonstrate the important part played by solubility-determined partitioning in separations on tightly cross-linked dextran gels.

INTRODUCTION

In ideal gel chromatography, the coefficient describing the partition between the mobile solvent phase and the gel phase is determined only by the change in configurational entropy corresponding to the free energy required to transport a solute molecule from the solvent to the gel phase. In polar systems, however, there may be interactions between the solute and gel phase characterized by an appreciable enthalpy term. In a previous communication[1], it was shown that in the case of tightly cross-linked dextran gels the separation is a function of both volume exclusion and partitioning determined by solubility behaviour, implying polar rather than steric interactions. The present investigation was undertaken to elucidate the relative magnitude of these effects as a function of temperature and solvent.

EXPERIMENTAL

The preparation of Sephadex G-15 columns was as previously described[2]. The dimensions are summarized in Table I. The non-aqueous solvents were reagent grade, dried over molecular sieves. The preparation and characterization of the cellodextrins and xylodextrins has been described elsewhere[1,2].

RESULTS AND DISCUSSION

Temperature dependence

In this section, the results are described in terms of K_{av} rather than K_D,

TABLE I

DIMENSIONS OF THE COLUMNS USED AT 25°

	DMSO	*Formamide*	*Water*	*0.1 M NaCl*
Settled bed volume (ml)	52.5	44.9	48.0	45.5
Void volume, V_0 (ml) (Blue Dextran)	26.4	19.8	19.4	19.0
Column height	66.9	57.2	61.1	58.0
Cross-sectional area (cm^2)	0.785			
Sample volume (ml)	0.1			
Sample concentration (mg/ml)	1			

as the former is more readily determined with precision when changes in the volume of the gel occur with variations in experimental conditions.

$$K_{av} = \frac{V_e - V_0}{V_T - V_0}; \qquad K_D = \frac{V_e - V_0}{V_I}$$

where V_T is the total volume and V_I the internal volume. For a discussion of these coefficients see, for example, ref. 3.

Fig. 1 shows that K_{av} increases with increasing temperature for the cellodextrins

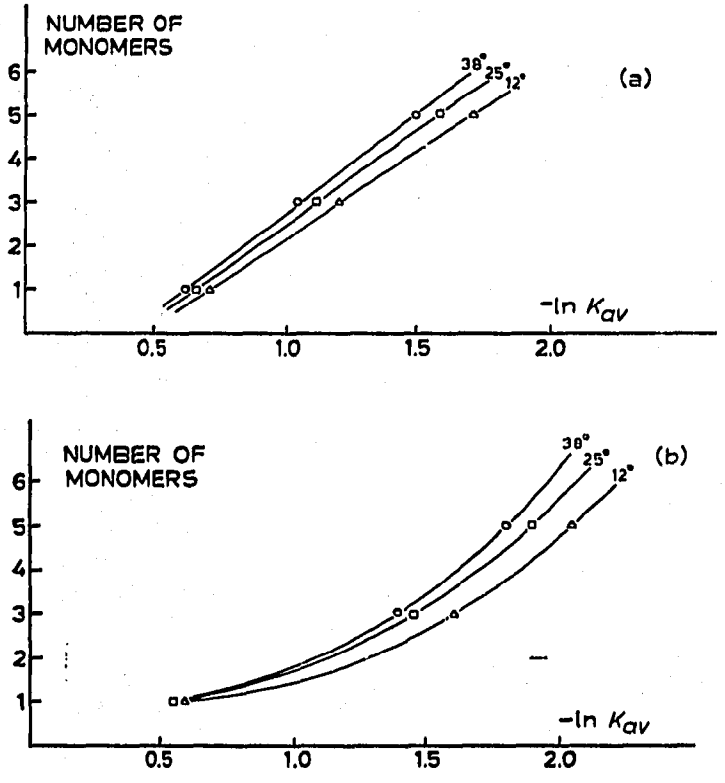

Fig. 1. Relationships at different temperatures between the number of chain units and $-\ln K_{av}$ for cellodextrins (a) and xylodextrins (b) on Sephadex G-15 (deionized water).

and xylodextrins on Sephadex G-15. Assuming equilibrium conditions, enthalpies may be determined from the dependence of ln K_{av} on temperature according to:

$$\Delta H^\circ = RT^2 \frac{\mathrm{d} \ln K_{av}}{\mathrm{d}T}$$

Free energies and entropies follow from:

$$\Delta G^\circ = -RT \ln K_{av}; \qquad \Delta S^\circ = \frac{(\Delta H^\circ - \Delta G^\circ)}{T}$$

The primary data are summarized in Table II. Fig. 2 shows the dependence of ln K_{av} on $1/T$. Values of ΔH°, ΔG° and ΔS° are given in Table III. With increasing temperature, the interactions between the solvent and the polysaccharide gel matrix decrease. This results in an increase in the activity of the solute in the gel phase (hence the positive sign of ΔH°) and consequently ΔH° will provide an index of the relative solubility behaviour of the solutes. The decreased interactions between

TABLE II

PARTITION COEFFICIENT, K_{av}, AS A FUNCTION OF TEMPERATURE FOR OLIGOSACCHARIDES ON SEPHADEX G-15 (DEIONIZED WATER)

	12°		25°		38°	
	K_{av}	$-\Delta ln\, K_{av}$	K_{av}	$-\Delta ln\, K_{av}$	K_{av}	$-\Delta n\, K_{av}$
Cellodextrin						
Glucose	0.48_8	0.48_7	0.51_3	0.44_8	0.53_0	0.40_9
Cellotriose	0.30_0	0.50_3	0.32_8	0.47_0	0.35_3	0.42_6
Cellopentaose	0.18_1		0.20_5		0.22_5	
$(V_T - V_0)$ (ml)	28.5		28.5		28.2	
Xylodextrin						
Xylose	0.54_8	1.0_0	0.56_8	0.89_5	0.56_8	0.83_6
Xylotriose	0.20_1	0.46_0	0.23_3	0.43_3	0.24_3	0.39_8
Xylopentaose	0.13_0		0.15_1		0.16_6	
$(V_T - V_0)$ (ml)	28.4		28.4		27.4	

TABLE III

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

	K_{av} (25°)	ΔH° ($cal \cdot mole^{-1}$)	ΔG° ($cal \cdot mole^{-1}$)	ΔS° ($cal \cdot mole^{-1}\ deg^{-1}$)
Cellodextrin				
Glucose	0.51_3	560	395	0.6
Cellotriose	0.32_8	1090	660	1.4
Cellopentaose	0.20_5	1450	940	1.7
Xylodextrin				
Xylose	0.56_8	265	335	−0.2
Xylotriose	0.23_3	1440	860	1.8
Xylopentaose	0.15_1	1660	1120	1.8

solvent and polysaccharides at higher temperature are evidenced by their well known large negative temperature coefficients of viscosity (due to decreased solvation) and their characteristic exothermic heats of dilution.

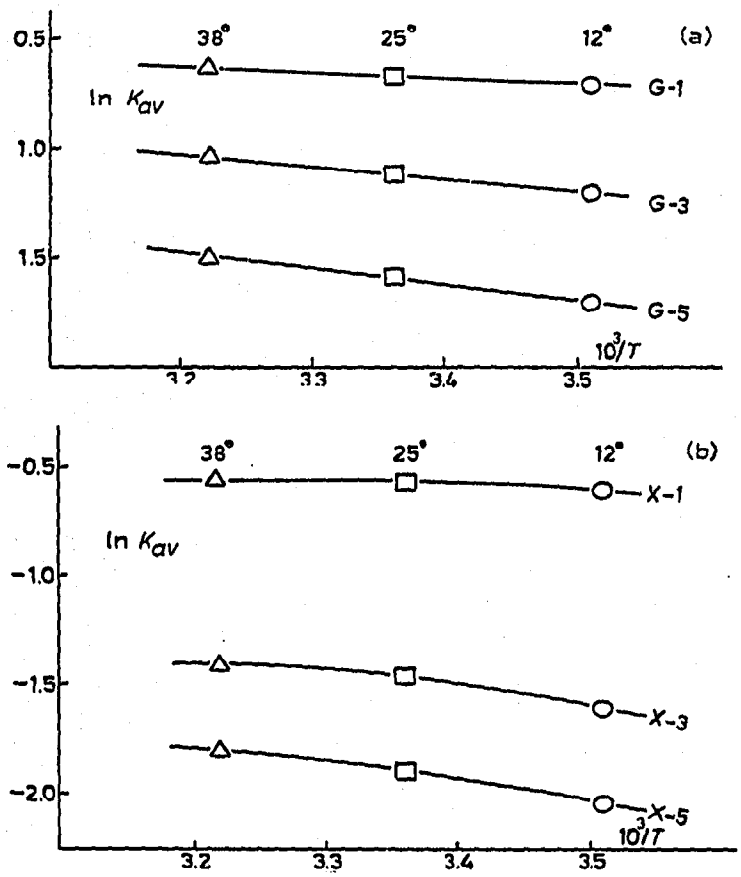

Fig. 2. Dependence of ln K_{av} on $1/T$ for cellodextrins (a) and xylodextrins (b) on Sephadex G-15 (deionized water). The increasing slopes suggest positive enthalpies increasing as the molecular weight increases.

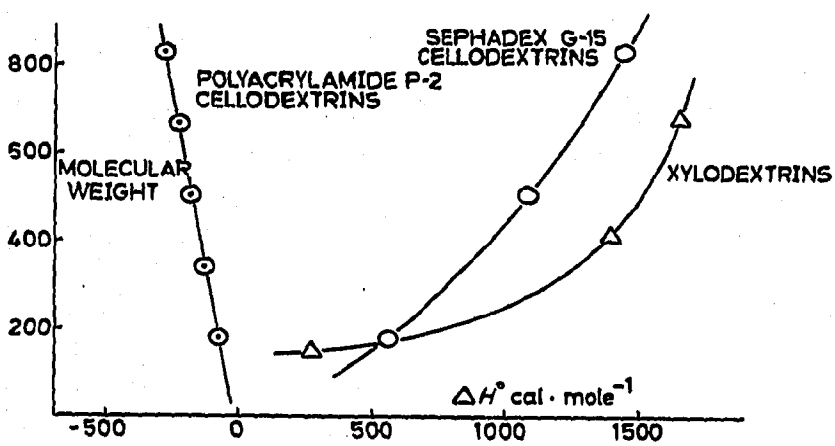

Fig. 3. Apparent enthalpies, reflecting the relative solubility behaviour of various oligosaccharides, as a function of molecular weight.

As previously noted[1], with dextran gels there is a solubility-determined partitioning of low-molecular-weight homologues between the gel and mobile solvent phases in addition to volume exclusion. The observed partition coefficient may thus be described as a function of $K_{\text{solubility partion}}$ and $K_{\text{volume exclusion}}$. As discussed below, the experimental evidence suggests that $K_{\text{volume exclusion}}$ is essentially independent of temperature for a given solute of the type considered here.

Fig. 3 shows that on Sephadex G-15, the relatively hydrophilic xylodextrins have a greater affinity for the solvent compared with the corresponding cellodextrins (with the exception of the monomers, as previously discussed[1]). The solubility behaviour of the oligosaccharides is not a simple function of their molar volumes, owing to the important part played by intramolecular hydrogen bonding. The extent of the latter may differ substantially for oligosaccharides of corresponding chain length but possessing only subtle differences in either conformation or structure. The upward curvature of the plots reflects the variation in solubility as a function of molecular weight. With polyacrylamide gels, the affinity of the solute for the gel increases linearly with molecular weight, suggesting an adsorption mechanism in this system[2].

Fig. 4 shows the correspondence between the separations with Sephadex G-15 and the relative solubility behaviour of the solutes. As would be expected, good correlation is also demonstrated in Fig. 5 between the separations in thin-layer chromatography (TLC) and $\Delta H°$.

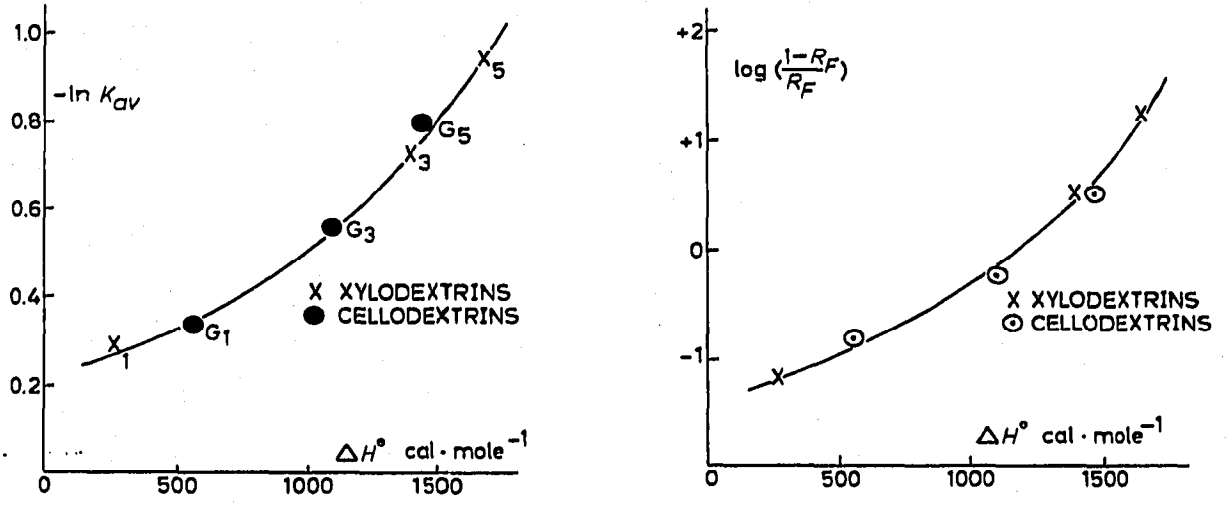

Fig. 4. Relationship between the separations of oligosaccharides on Sephadex G-15 and enthalpies reflecting their relative solubilities.

Fig. 5. Relationship between TLC data and enthalpies reflecting the relative solubility behaviour of various oligosaccharides.

It may be argued that the increasing partition coefficient with increasing temperature could be a consequence of the smaller hydrodynamic volume of the solute at the higher temperature. However, the partition coefficient shows a linear correspondence with the extended length of rigid rods, such as the oligosaccharides, in gel chromatography when solubility partitioning effects are absent[1]. Such behaviour has also been predicted by a statistical mechanical treatment[7]. The extended length of the cellodextrins is essentially invariant with temperature change[4] (at least up

to 70°). Furthermore, the observed partition coefficient does not correlate with the hydrodynamic volume of rod-like molecules in contrast to randomly coiling polymer chains. It may also be noted that the partition coefficient decreases with increasing temperature for the cellodextrins on polyacrylamide gels[2], whereas a smaller hydrodynamic volume would be expected to produce the opposite trend. Taken together, these observations suggest that $K_{\text{volume exclusion}}$ should exhibit little temperature dependence.

An alternative and more specific interpretation would be that the affinity of the less hydrophilic solutes for the dextran gel phase is due to adsorption. In this case, desolvation of the gel at the higher temperature promotes adsorption. Referring to Fig. 3, this would mean that the cellodextrins are more strongly adsorbed than the xylodextrins and that adsorption increases with molecular weight. The correlation of the TLC data with that from gel chromatography would simply mean that the tendency to adsorb is directly related to the decreasing solubility of the solute following Traube's law. Since both solubility partitioning and adsorption lead to a concentration of solute at the gel interface, the elucidation of such effects requires the use of an independent technique such as calorimetry. Preliminary micro-calorimetric results indicate a zero heat of mixing for cellopentaose, thus corresponding to solubility partitioning rather than adsorption since the accompanying heat of dilution is exothermic, reinforcing the heat of adsorption.

Solvent dependence

The dimensions of the Sephadex G-15 columns in some different solvents are given in Table I. As expected, the gel swells to an extent dependent on the polar nature of the medium (Table IV). The swelling is also reflected in differing void volumes (implying a deviation from spherical symmetry for the beads) which fall in the same order and are nearly in the same ratio as the swelling ratios given in the table.

TABLE IV

INTRINSIC VISCOSITIES OF GLUCOSE AND GEL SWELLING RATIOS IN DIFFERENT SOLVENTS

	$[\eta]_{25}$ $(ml \cdot g^{-1})$	*Gel swelling ratio*[a]	*Dipole moment, δ* $(cal \cdot g^{-1})$
DMSO	6.0_0	2.88	3.9
Formamide	4.0_0	2.62	3.25
Water[b]	2.68 (12°) 2.54 (25°) 2.36 (38°)	2.42	1.85
0.1 *M* NaCl	2.5_0	2.41	

[a] Ratio of swollen to dry volume.
[b] Interpolated from the results of IHNAT AND GORING[4].

Values of K_{av} are listed in Table V for the separations of the cellodextrins in the different solvents. Fig. 6 shows the relative magnitude of the solvent effect. The more pronounced the interactions between the solvent and the polymer constituting the gel matrix (falling in the order DMSO > formamide > water > 0.1 *M* NaCl), the lower is the activity of the solute in the gel phase, *i.e.* the smaller the partition coefficient.

TABLE V

PARTITION COEFFICIENT, K_{av}, FOR THE CELLODEXTRINS IN DIFFERENT SOLVENTS AT 25° (SEPHADEX G-15)

Cellodextrin	*Dimethyl sulphoxide*	*Formamide*	*Water*	*0.1 M NaCl*
G-1	0.341	0.500	0.513	0.528
G-2	0.190	0.376	0.415	0.427
G-3	0.104	0.293	0.328	0.336
G-4	0.058	0.213	0.259	0.272
G-5	0.025	0.144	0.205	0.215
G-6	Excluded	0.112	0.162	0.189

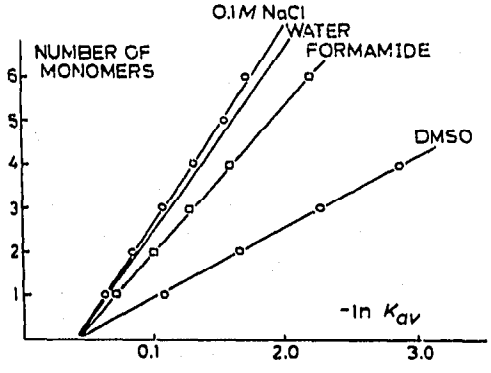

Fig. 6. Separations of the cellodextrins on Sephadex G-15 in different solvents.

Table IV lists intrinsic viscosities for glucose in DMSO, formamide and water and the dipole moments of these solvents. Since glucose may be approximated as a spherical molecule (axial ratio equals unity), the differences in intrinsic viscosity simply reflect the quantity of solvent bound to the molecule. The magnitude of the solute–solvent interactions thus increases in the order of the dipole moments of the latter and the swelling ratios for the gel matrix. However, the axial length of the solute is essentially independent of the degree of hydration[4]. Consequently, as in the case of temperature variation, $K_{\text{volume exclusion}}$ should be nearly independent of the solvent also, since the extended length is the parameter determining the elution volume in gel chromatography when solubility-partitioning effects are absent. The solvent effect may thus also be assigned to $K_{\text{solubility partition}}$, *i.e.* the better the solvent, the more the partition coefficient is changed in its favour relative to the gel phase. These results conform to the finding of ISHERWOOD AND JERMYN[8] that for the separation of oligosaccharides in TLC, the partition coefficient is a linear function of log N, where N is the mole fraction of water in the developing solvent.

The interpretation given above is necessarily qualitative and oversimplified. The real situation may well be more complex than visualized. For example, $K_{\text{volume exclusion}}$ may be expected to show some degree of solvent dependence, particularly with associated solvents such as DMSO and formamide. Nevertheless, this should not invalidate the conclusion that solubility partitioning is the major part of the temperature and solvent effects in tightly cross-linked dextran gels.

One may conclude that when the solutes to be separated have differing solubility behaviour as characterized by large excess heats of mixing (*i.e.* the interactions are polar in nature) solubility-determined partitioning will always occur together with partitioning controlled by volume exclusion. The extent to which solubility partitioning is important will depend on the magnitude of the solvent–gel matrix interactions.

Adsorption effects will occur when the solute or gel matrix possess functional groups which can interact specifically with each other. The latter forms a special case on the generally occurring partition phenomenon. As examples one may give the interactions of urea and formamide with dextran gels[5] and the adsorption phenomena encountered with oligosaccharides on cellulose gels[6]. In the latter, the primary alcohol groups apparently facilitate the adsorption of oligosaccharides. In contrast, the adsorption of such compounds to dextran gels apparently does not occur owing to the participation of this group in the main dextran structure (α-(1 → 6) links).

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