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GEL CHROMATOGRAPHY: THE EFFECT OF TEMPERATURE ON PARTITIONING

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SUMMARY

The influence of temperature on the partitioning of different homologous series in cellulose and cellulose acetate gels in the solvents water and dimethylformamide is described. The complex nature of the interactions in these and other systems is discussed and possible explanations are given for the thermodynamic quantities evaluated from the partitioning data.

INTRODUCTION

In a gel chromatographic system there are invariably intermolecular forces (although not necessarily specific, *e.g.*, hydrogen bonding) between the components, *i.e.*, gel matrix, solvent and solute. Gel and solute, for example, must always swell and dissolve, respectively, in the medium. The magnitude of these interactions will depend on the concentrations of gel matrix (cross-link density) and of solute in the medium. In systems in which solute–gel interactions are sufficiently strong (usually either through hydrogen bonding or π-bonding) so that the partition coefficient exceeds unity, it has been customary to use the term "adsorption effects". It will be appreciated, however, that a partition coefficient of less than unity does not infer negligible solute–matrix interactions.

As an approach to obtaining a better insight into the magnitude of such interactions, the effect of temperature has been studied with cellulose and cellulose derivative gels. These gels are suitable as the character of the matrix is easily modified by substituting with an appropriate functional group. This paper compares results obtained with a cellulose gel and a highly acetylated form of the same material. Solutes (homologous series) varying in both chemical nature and molecular size have been used. Gel chromatographic experiments were performed at different temperatures in water as a polar hydrogen-bonding solvent and in dimethylformamide (DMF) as a weakly polar, aprotic solvent.

EXPERIMENTAL

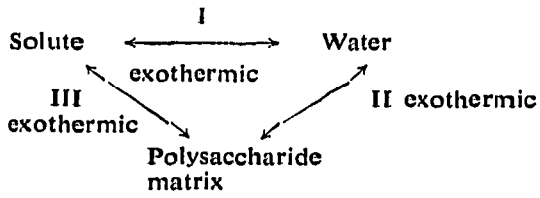
The preparation and properties of the cellulose and cellulose acetate gels have been described earlier[1,2]. Details concerning the solutes have also been given[2], and the experimental arrangement has been described[3].

RESULTS AND DISCUSSION

Prior to discussing the relative magnitudes of the thermodynamic parameters for specific solutes, some general remarks concerning the signs to be expected for the enthalpies in aqueous media are appropriate.

Polysaccharide gels with aqueous solvents

As specific interactions (hydrogen bonding) occur between the components in these systems, some qualitative conclusions can be drawn regarding the enthalpies determined by chromatographic experiments at different temperatures. As pointed out previously[4], the thermodynamic parameters are the net result of the several contributing interactions, *viz.*, the temperature dependence of the retention volume does not only reflect the solute–gel interaction but depends on the sum of the interactions between the three components of the system. The heats of mixing with water are typically exothermic for hydrogen bonding solutes (the solubility decreases with increasing temperature) and polysaccharide matrix (the swelling decreases with increasing temperature).



With weakly polar solutes interacting weakly with the matrix (for example, sugars and alcohols), interactions I and II predominate. Thus when de-solvation occurs with increasing temperature, the simultaneously decreasing solute-solubility causes the partitioning to favour the matrix, *i.e.*, the retention volume is found to increase with temperature in spite of the exothermic character of III. This tendency will clearly be enhanced with increasing molecular weight. The observed trend in the change in retention volume with temperature can be expressed as positive enthalpies as derived from the equation

$$\frac{d\ln K}{dT} = \frac{\Delta H^0}{RT^2} \qquad (1)$$

In accordance with the above, it has been shown that the magnitude of ΔH° is related to solubility behaviour —the larger is ΔH°, the lower is the solubility; thus it was found that the enthalpies derived from the temperature dependence of partitioning for oligosaccharides on dextran gels are simply related to their separations in liquid–liquid chromatography[5].

The ability of polysaccharides to solvate extensively in aqueous media most probably derives from the excellent fitting of sugar structures into the water lattice[6]. De-solvation of the swollen matrix can be expected to increase the interactions with solutes as the gel hydroxyl groups become increasingly accessible when the temperature is raised. This is supported by the finding that the addition of simple salts, which also leads to de-solvation, causes increased retention volumes[5]. Thus the positive

enthalpies are essentially an artefact deriving from the change in the character of the solvent-swollen matrix.

It appears confusing to invoke "hydrophobic interactions" between lipophilic groups in the gel (the cross-links in dextran gels) to explain the well known phenomenon of de-solvation of polysaccharides with increased temperature[7]. Even a column of non-cross-linked cellulose (or dextran) will exhibit a similar decrease in volume.

It is also questionable, in the light of the above, whether one should regard the partitioning of weakly polar solutes in such gels as entropy-determined[7,8], *i.e.*, essentially a "hydrophobic interaction". Although the thermodynamic parameters themselves cannot provide an unambiguous answer, this would assume that alterations in solvent arrangement dominate the thermodynamic parameters. This assumption may not be justified, however, as the qualitatively well understood changes in solvent–gel (solvation), solute–solvent (solubility) and solute–gel (adsorptive) interactions must be taken into account and in fact can give an adequate explanation of the effect. Also, the finding that an eluent such as formamide, or the addition of the hydrogen-bond breaking solute guanidinium hydrochloride to the aqueous medium, causes substantial decreases in the thermodynamic parameters, follows directly from the absence or diminution of the solvation layer characterizing the interactions of water with polysaccharide chains.

With solutes that are capable of interacting strongly with the gel matrix (for example, urea and thiourea[9], and halogenated phenols[10]) the net enthalpies are reversed in sign because the exothermic solute–gel interactions (III, see above) are now dominant. An increase in temperature will consequently result in decreased retention volumes (ΔH° negative according to eqn. 1). Even with weakly interacting solutes, it has been shown by direct microcalorimetric measurements[11] that the solute–gel (in this case oligosaccharides on dextran gels) interaction III is exothermic (ΔH° negative). Thus ΔH° values as a function of molecular size will reflect solute–solubility behaviour as modified by solute–gel interactions or *vice versa*.

It may be noted that with polyacrylamide gels in water, the solvent–gel matrix interactions are typically endothermic[3,4,12]. Consequently, an increase in temperature will result in decreasing retention volumes (ΔH° negative) when either weakly or strongly interacting solutes are chromatographed. This behaviour is opposite to that for polar solutes interacting weakly with a polysaccharide matrix.

Cellulose gel with water

*Cellodextrins**. The experimental data for this system are presented in Table I and the derived thermodynamic parameters in Table II. Previously reported net enthalpies for this homologous series on dextran[5] and hydroxyethylcellulose[13] gels swollen in water are positive and increase monotonically as a function of molecular size. As explained above, this is primarily due to the manner in which solute–solvent interactions decrease with increasing molecular weight. The situation is similar but more complicated with cellulose gels because, firstly, solute–gel interactions are considerably stronger and, secondly, the reactivity of the matrix polymer increases with increasing temperature as strained inter- and intramolecular hydrogen bonds rupture. Fig. 1 shows the temperature dependence of the partition coefficient as a function of molecu-

* This article deals with glucose (G1), cellobiose (G2), cellotetraose (G4) and cellopentose (G5).

TABLE I

PARTITION COEFFICIENTS, K_{av}, DETERMINED AT DIFFERENT TEMPERATURES

$K_{av} = \frac{V_e - V_o}{V_T - V_o}$, where V_o = void volume, V_T = total volume of swollen gel, and V_e = elution volume for a given solute. System: cellulose–water.

Solute	*12°*	*20°*	*28°*	*36°*
G1	0.95_9	0.99_0	1.0_0	1.0_3
G2	0.92_0	0.95_8	0.98_5	1.0_1
G4	0.98_2	0.98_0	1.0_1	1.0_3
G5	0.98_2	0.98_0	1.0_5	1.1_0
Ethylene glycol	0.96_8	0.97_5	0.98_3	1.0_0
Erythritol	0.98_4	0.96_5	1.0_0	0.97_0
Mannitol	0.96_8	0.96_5	0.98_5	0.94_5

TABLE II

THERMODYNAMIC PARAMETERS FOR CELLODEXTRINS AND POLYHYDRIC ALCOHOLS IN THE CELLULOSE GEL-WATER SYSTEM AT 25°

Solute	K_{av}	$\Delta H°$ (*cal·mole*$^{-1}$)	$\Delta G°$ (*cal·mole*$^{-1}$)	$T\Delta S°$ (*cal·mole*$^{-1}$)
G1	1.0_0	+ 530	0	+ 530
G2	0.97_1	+ 590	+17	+ 575
G4	1.0_0	+ 480	0	+ 480
G5	1.0_2	+1200	+10	+1190
Ethylene glycol	0.98_2	0	+11	− 11
Erythritol	0.97_8	0	+13	− 13
Mannitol	0.96_6	0	+21	− 21

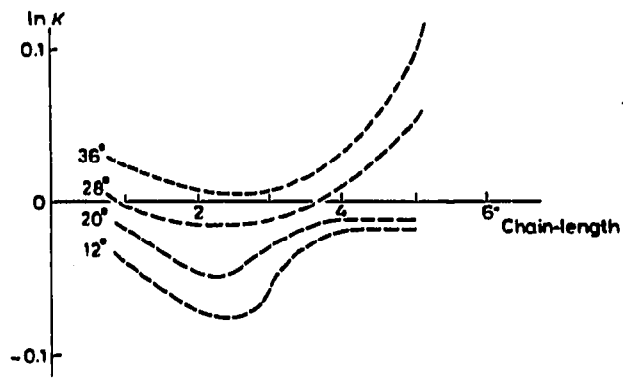


Fig. 1. Temperature dependence of the partition coefficients for the cellodextrins on the cellulose gel.

lar size in such a system. At the lower temperatures (12° and 20°), there is an initially increasing exclusion with increasing size; thereafter, the solute–gel interactions become dominant with a limiting capacity to interact at the tetramer/pentamer. At the higher temperatures (28–36°), there is a continuous increase in solute–gel interactions owing to the greater number of hydroxyl groups that are available for interaction in this temperature interval. This finding corresponds to the now well documented second-order transition that occurs with both dry and water-swollen cellulose at a temperature of about 25° (see, for example, refs. 14–17). This transition has been attributed to the breaking of weak hydrogen bonds and/or changed perturbations of the water structure.

As a consequence of the several competing interactions and the changing character of the matrix as a function of temperature, a monotonic change in $\Delta H°$ with molecular size is hardly to be expected (see the values in Table II).

Polyhydric alcohols. With these compounds, there is no detectable influence of temperature on the retention volume. Likewise, there is no appreciable dependence of the partition coefficient on molecular size at a given temperature. This is presumably

TABLE III

PARTITION COEFFICIENTS, K_{av}, DETERMINED AT DIFFERENT TEMPERATURES

Solute	*12°*	*20°*	*28°*	*36°*
System: cellulose–DMF				
G1	0.98_0	0.96_8	0.95_7	0.94_0
G2	0.95_5	0.94_7	0.92_8	0.91_0
G4	0.93_5	0.89_7	0.85_2	0.84_2
Ethylene glycol	0.95_0	0.92_0	0.90_8	0.87_4
Erythritol	1.00_0	0.98_0	0.96_7	0.94_0
Mannitol	1.05_2	1.03_0	1.00_7	0.98_2
G1Ac	0.80_5*	0.80_5	0.79_7	0.77_2
G2Ac	0.77_2*	0.76_4	0.75_0	0.72_1
G4Ac	0.71_2*	0.70_2	0.69_7	0.69_8
Toluene	0.87_0*	0.86_4	0.86_4	0.85_5
Methyl-2-oligophenylene	0.84_5*	0.84_0	0.82_5	0.82_0
System: cellulose acetate–DMF				
G1	–	0.79_0	0.78_5	0.77_1
G2	–	0.72_8	0.72_0	0.70_7
G4	–	0.61_8	0.62_5	0.60_3
Ethylene glycol	–	0.86_0	0.86_1	0.85_0
Erythritol	–	0.82_8	0.82_0	0.81_0
Mannitol	–	0.79_0	0.78_5	0.78_0
G1Ac	0.84_8	0.81_0	0.80_1	–
G2Ac	0.77_4	0.76_2	0.75_5	–
G4Ac	0.63_0	0.62_5	0.62_1	–
Toluene	0.99_1	0.94_6	0.93_0	–
Methyl-2-oligophenylene	0.89_6	0.88_6	0.88_0	–

* Values at 15°.

TABLE IV

THERMODYNAMIC PARAMETERS FOR VARIOUS SOLUTE HOMOLOGOUS SERIES IN THE SYSTEMS CELLULOSE GEL–DMF AND CELLULOSE ACETATE GEL–DMF AT 25°

Solute	*Cellulose gel–DMF*				*Cellulose acetate gel–DMF*			
	K_{av}	$\Delta H°$ ($cal \cdot mole^{-1}$)	$\Delta G°$ ($cal \cdot mole^{-1}$)	$T\Delta S°$ ($cal \cdot mole^{-1}$)	K_{av}	$\Delta H°$ ($cal \cdot mole^{-1}$)	$\Delta G°$ ($cal \cdot mole^{-1}$)	$T\Delta S°$ ($cal \cdot mole^{-1}$)
Cellodextrin								
G1	0.95_9	−260	+ 25	−320	0.79_1	−440	+135	−575
G2	0.93_3	−455	+ 40	−365	0.72_3	−270	+190	−460
G4	0.87_8	−830	+ 75	−905	0.61_5	−130	+285	−415
Polyhydric alcohol								
Ethylene glycol	0.91_3	−480	− 10	−470	0.86_6	−230	+ 85	−315
Erythritol	0.97_3	−505	+ 15	−520	0.82_5	−160	+110	−270
Mannitol	1.02	−560	+ 55	−615	0.78_8	−120	+140	−260
Cellodextrin acetate								
G1Ac	0.80_0	−110	+130	−240	0.81_5	−660	+120	−780
G2Ac	0.76_2	−230	+160	−390	0.76_7	−265	+155	−420
G4Ac	0.70_8	−355	+205	−560	0.62_0	−180	+280	−460
p-Oligophenylene								
Toluene	0.86_4	− 90	+ 85	−175	0.94_3	−545	+ 35	−580
Methyl-2-oligophenylene	0.83_4	−265	+105	−370	0.88_7	− 80	+ 70	−150

due to the approximate equivalence of solute–solvent and solute–gel interactions with these highly hydroxylated compounds, which, moreover, should have nearly constant polarities as a function of size.

Cellulosic gels with DMF

The data for these systems are presented in Tables III and IV. In contrast to the results with polysaccharide gels in water, the swelling of the cellulose gel is observed to increase with increasing temperature when DMF is used as the solvent. The inflection in Fig. 2 at 24° corresponds to the second-order transition referred to above. This transition must reflect a change in matrix structure. The disrupted hydrogen bonds become effectively "labelled" by stronger bonding with DMF molecules which inhibits their re-formation. Such an increase in swelling is not observed with water as solvent because hydroxyl–water interactions are comparatively weak and there is a rearrangement of hydrogen bonds leading to a more stable, compact structure with increasing temperature. However, in the presence of a solute that is capable of interacting strongly with these sites, the rearrangement is hindered by the formation of hydroxyl–solute bonds, *i.e.*, adsorption increases with increasing temperature. As noted in Fig. 2, maximum swelling is found at approximately 40°. This appears to be anomalous because the gel–solvent interaction is expected to be exothermic, as established by studies of the adsorption of DMF vapour on a cellulose surface[18]. Two possibly contributory reasons may be put forward: (a) exothermic interactions at high matrix concentrations may reverse to endothermic at higher dilutions, *i.e.*, the fully solvated polymer constituting the gel may mix endothermally with solvent; and (b) additional interactive sites (the hydroxyl groups on the gel) become available in cellulose on raising the temperature, *i.e.*, inter- and intramolecular hydrogen bonds in strained configurations rupture and will interact preferentially with additional solvent molecules.

The second factor is favoured, because it was found that the acetylated gel (see also below) exhibits no temperature dependence of swelling over the same interval. In either case, the result will be to favour a lower retention volume of the solute with increasing temperature (negative enthalpies). With weakly interacting solutes, the

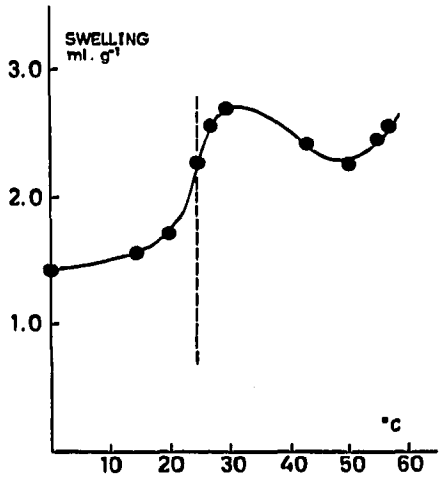


Fig. 2. Swelling of the cellulose gel in DMF as a function of temperature.

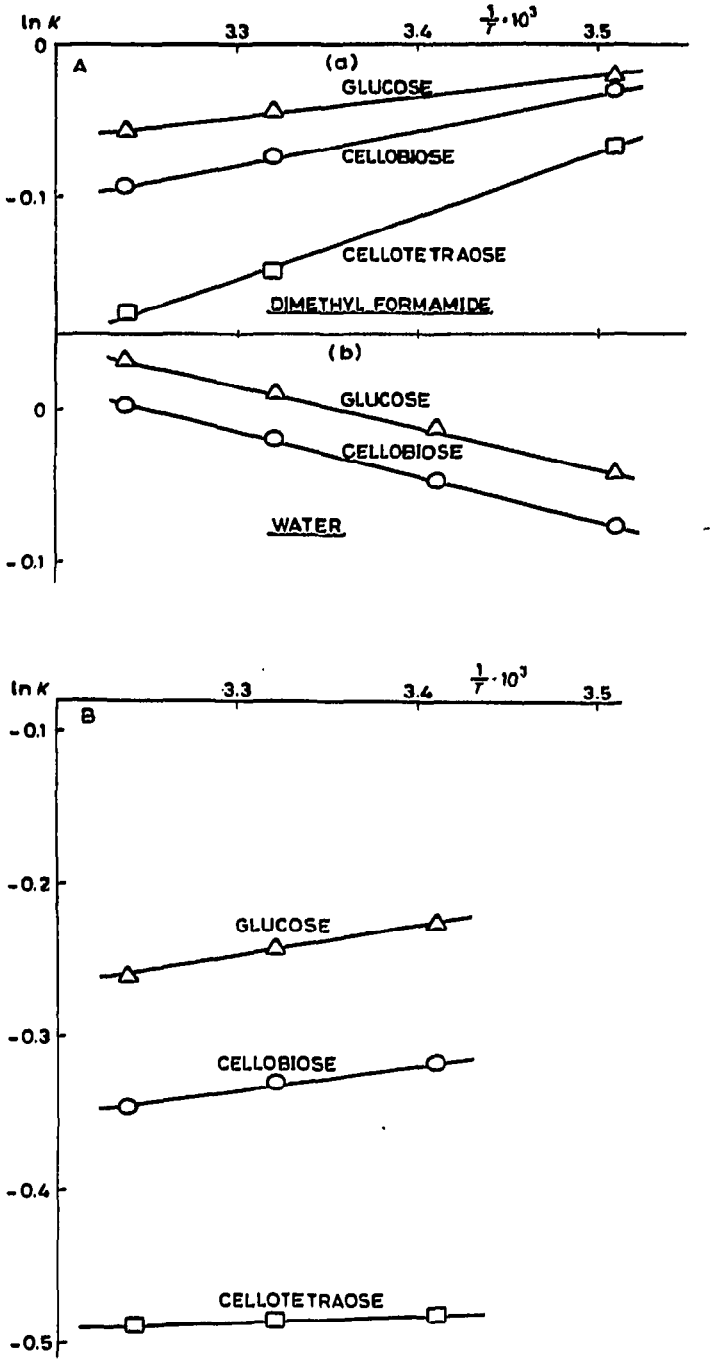


Fig. 3. (A) Temperature dependence of the partition coefficient for cellodextrins on the cellulose gel in DMF (a) and water (b). Positive slopes correspond to negative enthalpies and *vice versa*. (B) Temperature dependence of the partition coefficient for cellodextrins on the cellulose acetate gel in DMF. The enthalpy parameter decreases with increasing solute size.

solvent–gel interaction should be dominant and lead to smaller solute retention volumes with increasing temperature. Similarly, with strongly interacting solutes, the solute–gel interaction (III, see above) will concomitantly decrease with increasing temperature. The difference in the thermodynamic parameters between successive members of a homologous series on a given gel will reflect the sum of solute–solvent and solute–gel interactions, which have opposite effects on the partitioning.

We now proceed to discuss specific solutes partitioning in the cellulose and cellulose acetate gels with DMF as the eluent. The following is essentially a qualitative discussion of the enthalpy parameter, although entropies and free energies are included in the tables. It may be noted that both $\Delta H°$ and $T\Delta S°$ are negative regardless of solute type in DMF. This is a result of the dominating role of gel–solvent interactions as concluded in the preceding discussion. Furthermore, non-polar compounds interact most strongly with the acetate gel and polar compounds least; the opposite is true for the cellulose gel.

Cellodextrins. The temperature dependences for these compounds on the cellulose and cellulose acetate gels are depicted in Fig. 3. With the cellulose acetate gel, the solute–gel interactions are most pronounced with the lowest-molecular-weight compound and decrease as the solute is progressively excluded from the gel with increasing size. This is the expected trend and at a sufficiently high molecular weight the separation according to molecular size should be determined solely by steric factors.

With the cellulose gel, the situation is reversed, the solute–gel interactions increase with solute size. This effect is pronounced with the cellodextrins because these compounds can interact favourably with the matrix, especially as the solute solubility decreases with increasing molecular weight. The net enthalpy parameter consequently has a progressively negative value.

Polyhydric alcohols. As found for the cellodextrins, these compounds interact more strongly with the cellulose gel than with the acetylated gel. In both instances,

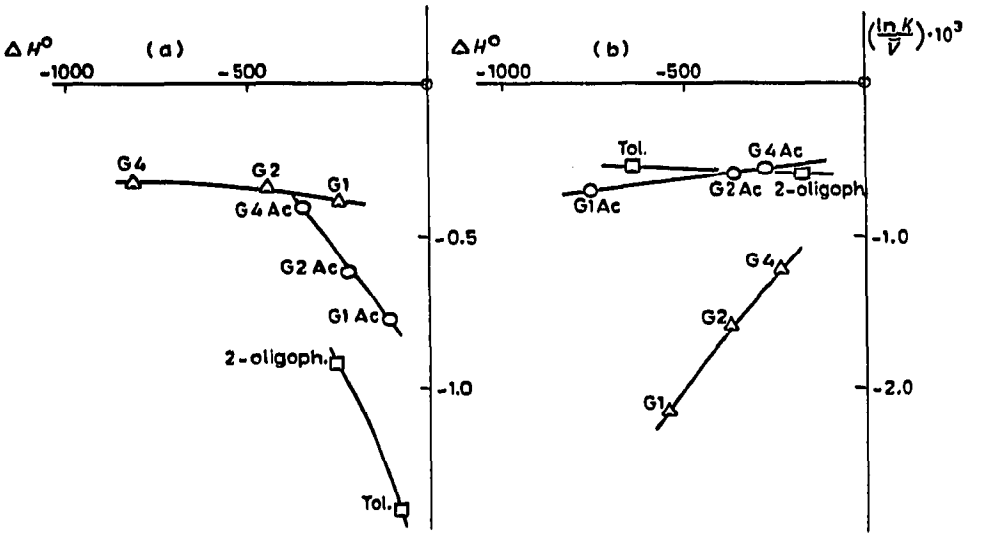


Fig. 4. The enthalpy parameter, $\Delta H°$, as a function of $\ln K/\bar{V}$, where $\bar{V}$ is the solute partial molar volume, for various solutes on the cellulose gel (a) and the cellulose acetate gel (b). 2-Oligoph. = methyl-2-oligophenylene, Tol. = toluene.

however, there is a negligible molecular-weight dependence of the enthalpies. This is because there is only a slight variation in solute character between successive members of this homologous series, which differ only by a –CHOH group.

Cellodextrin acetates. As with the cellodextrins, the interaction of the acetates with the acetylated matrix is most pronounced at the lowest molecular weight. Furthermore, glucose acetate has a greater net enthalpy than glucose.

With the cellulose gel, the interactions increase with increasing solute size and the enthalpies are lower with the acetates than with the corresponding cellodextrins. In addition, the weak solute–matrix interactions modify the relatively strong solvent–cellulose gel interaction to an extent proportional to solute size, *i.e.*, there is a competitive interaction between solute and solvent for the gel. Thus the fundamental difference in partitioning behaviour between these two gels stems from the strong solvent–gel interaction in the case of the cellulose gel.

Toluene and methyl-2-oligophenylene. Toluene interacts most strongly with the cellulose acetate gel (in fact, at a loading of 3.5 mg, the interaction is apparently irreversible) owing to its basic nature. As for the other solutes dealt with above, the situation is reversed with the cellulose gel.

Fig. 4 shows $\Delta H°$ as a function of ln K normalized with respect to the partial molar volume of the solute[19]. With the cellulose gel, $\Delta H°$ increases with molecular size with each solute type, while with the acetylated gel the reverse is true. The exclusion tendency, reflected in ln $K/\overline{V}$, for the solutes is also opposite for the two gels (*cf.* cellodextrins and cellodextrin acetates).

Fig. 5 shows $\Delta H°$ plotted against ln K for different solutes. The order for interaction with the cellulose gel is mannitol > glucose > glucose acetate, and this order is

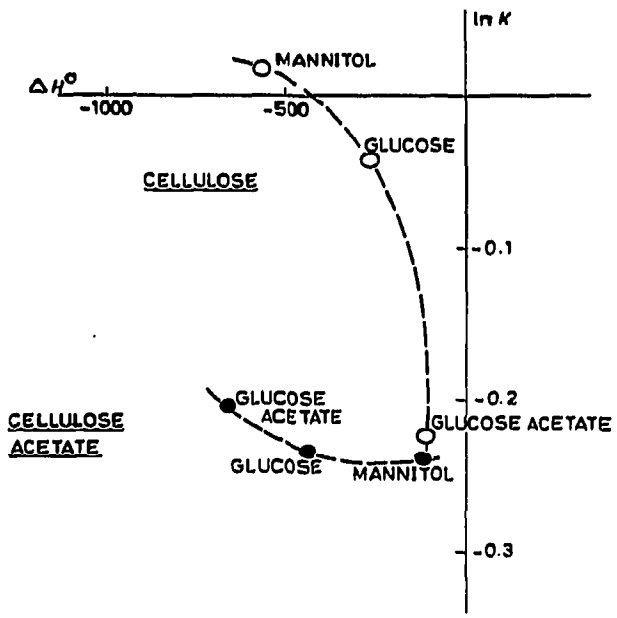


Fig. 5. $\Delta H°$ *versus* ln K for different solutes on cellulose and acetylated cellulose gels.

reversed on the acetylated matrix. As would be expected, the exclusion contribution in $\ln K$ is inversely related to $\Delta H°$.

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REFERENCES

1 K. Chitumbo and W. Brown, *J. Polym. Sci., Part C*, 36 (1971) 279.
2 K. Chitumbo and W. Brown, *J. Chromatogr.*, 80 (1973) 187.
3 W. Brown, *J. Chromatogr.*, 52 (1970) 273.
4 W. Brown, *Chem. Scr.*, 2 (1972) 25.
5 W. Brown, *J. Chromatogr.*, 59 (1971) 335.
6 M. A. Kabayoma and D. Patterson, *Can. J. Chem.*, 36 (1958) 563.
7 K. Lampert and H. Determann, *J. Chromatogr.*, 56 (1971) 140; 69 (1972) 123.
8 N. V. B. Marsden, *Proc. 1st European Biophysics Congr., Baden, Austria, 1971.*
9 N. V. B. Marsden, *Ann. N.Y. Acad. Sci.*, 125 (1965) 478.
10 A. J. W. Brook and K. C. Munday, *J. Chromatogr.*, 51 (1970) 307.
11 W. Brown and J. Öhman, *LKB Products Application Note*, Stockholm, 1972.
12 H. W. Dellweg, M. John and G. Trenel, *J. Chromatogr.*, 57 (1971) 89.
13 W. Brown and K. Chitumbo, *Chem. Scr.*, 2 (1972) 88.
14 Y. Ishida, M. Yoshino, M. Takayanagi and F. Irie, *J. Appl. Polym. Sci.*, 1 (1959) 222.
15 M. V. Ramiah and D. A. I. Goring, *J. Polym. Sci., Part C*, 11 (1965) 27.
16 M. Wahba, *Ark. Kemi*, 29 (1968) 395.
17 M. Kimura, H. Hatakeyama, M. Usuda and J. Nakano, *J. Appl. Polym. Sci.*, 16 (1972) 1749.
18 E. A. Colombo and E. H. Immergut, *J. Polym. Sci., Part C*, 31 (1970) 137.
19 S. Hjertén, *J. Chromatogr.*, 50 (1970) 189.