GEL CHROMATOGRAPHY: INTERACTIONS BETWEEN GEL MATRIX, SOLVENT AND SOLUTE

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SUMMARY

The partitioning behaviour of homologous series of low-molecular-weight polar and non-polar compounds has been studied on gels of cellulose and various derivative forms in dimethylformamide. The nature of the functional groups present in the gel matrix, solute and solvent markedly affects the solute partitioning. The observed partitioning is discussed in terms of the structures of the components.

INTRODUCTION

Previous papers have dealt with the influence of the chemical natures of the gel matrix and the solvent on the partitioning of solutes in gel chromatography[1-3]. The characteristics of the functional groups of the components determine their capacity for mutual interaction. These effects are pronounced with low-molecular-weight solutes, particularly when two or more of the components can interact specifically through hydrogen bonding. As a route to obtain a better understanding of the importance of such interactions and their eventual utilization in gel chromatography, partitioning experiments have been performed using cellulosic gels. Cellulose is particularly convenient as the character of the gel matrix can be simply modified by substitution with an appropriate functional group. This paper describes the results obtained with gels of cellulose and its acetate, methyl and trimethylsilyl derivatives.

Homologous series of solutes were chosen that differed widely in character and covered the molecular weight range from 50 to 1500. Column chromatography experiments were performed mainly in the weakly polar, aprotic solvent dimethylformamide (DMF). The latter was selected because it permitted comparisons in a common solvent in which both polar and non-polar gels and solutes swell and dissolve, respectively. Results were obtained using water and dimethyl sulphoxide in some systems.

Obviously, it is the resultant of the interactions (both energetic and steric) between the three components that determines the magnitude of the partition coefficient measured in a gel chromatography experiment. However, from a knowledge of the interactions to be expected between the functional groups and recent progress with aprotic solvents[11], it is possible to suggest explanations for the observed partitioning phenomena. The terms "acidic" and "basic" are used here in the Lewis

sense[4]; for example, the electrophilic carbon atom of a carbonyl group imparts "acidic" character, while the nucleophilic nitrogen atom in DMF imparts "basic" character.

The swelling of the various gel types is of interest; a low degree of swelling is observed with the gels of cellulose and its O-trimethylsilyl derivative and a high degree of swelling with gels of the O-methyl and acetate derivatives. With cellulose itself, inter- and intramolecular hydrogen bonding limits the interaction with solvent molecules and swelling, as would be expected, increases with increasing temperature as such bonds become less frequent. The low degree of swelling of the gel of the trimethylsilyl derivative, in comparison with the chemically similar gel of the methyl derivative, is the result of the sterically limited access of solvent to the ether oxygen sites. As neither hydrogen bonding nor steric hindrance interferes with the interaction of the solvent with the ether oxygen of methyl cellulose or the carbonyl group of cellulose acetate, the degree of swelling of both of these gels is high.

EXPERIMENTAL

Preparation of gels

Cellulose gel. The preparation of this material (C-80) has been described in detail elsewhere[5]; the degree of swelling was 2.7 ml/g in DMF. Material from the same batch was used for preparing the derivative gels described below.

Acetylation. Cellulose gel C-80 (20 g) was allowed to swell overnight in pyridine that had previously been dried over molecular sieves. Acetylation was carried out in a 1:1 mixture (by volume) of dry pyridine and acetic anhydride (300 ml total volume) at 60° for 12 h. The acetylated gel was washed separately with methanol, water and acetone and finally vacuum-dried at room temperature. The degree of acetylation was 1.55 and swelling 8.8 ml/g in DMF.

Methylation. Cellulose gel C-80 (20 g) was dried overnight under vacuum at 50°. This material was placed in a two-necked 2-l flask and 400 ml of 20% KOH solution added. The mixture was stirred slowly under nitrogen for 2 h. Dimethyl sulphate (100 ml) was slowly added over 6–8 h with gentle stirring and the reaction mixture was maintained under an atmosphere of nitrogen for 24 h. The methylated material was filtered off, washed successively with water, ethanol, water again and finally with 20% KOH solution. The above procedure was repeated twice more so as to increase the degree of methylation. The product was washed with water, neutralized to pH 6–7 and finally washed exhaustively with acetone, followed by vacuum-drying at 40°. The degree of methylation was 1.89 and swelling 9.8 ml/g in DMF.

Trimethylsilanization. Cellulose gel C-80 was dried under vacuum at 50°, following exhaustive washing with dry methanol. This material (20 g) was placed in a three-necked 1-l round-bottomed flask containing 200 ml of dry pyridine and 200 ml of dry toluene. Trimethylchlorosilane (63 g) was added dropwise with gentle stirring at room temperature and the reaction mixture was slowly heated to 80° with stirring. After 2 h, the mixture was cooled to room temperature and 100 ml of dry methanol added. The product was filtered off, washed with 200 ml of 2% sodium acetate in methanol and finally with 300 ml of methanol. The product

was dried under vacuum at 25°. The degree of substitution was 2.72 and swelling 4.4 ml/g in DMF.

Solutes and solvents

The polyethylene oxides and polyhydric alcohols were commercially available materials (Fluka AG, Buchs, Switzerland). The molecular weights of the polyethylene oxides have been reported previously[1]. The cellodextrins were prepared according to Brown[6]. Methyl *p*-oligophenylenes* were synthesized as described by Claesson *et al.*[7]. Details concerning the cellodextrin acetates have been described previously[2]. D-Glucose penta-acetate was obtained from Fluka AG.
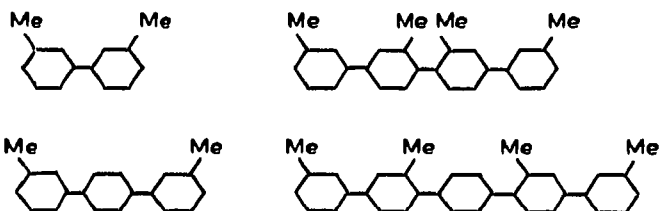
Methyl *p*-oligophenylenes

The solvents were of analytical-reagent grade.

Column preparation and operation

The techniques used in the preparation of the column and the instrumental arrangement have been described in detail by Brown[6].

Except when otherwise stated, the sample loading was 2 ml of 7 g/l solution. This relatively high loading was used so as to enhance solute interactions.

RESULTS AND DISCUSSION

The results are presented as graphs of ln K_{av} *versus* partial molar volume. Negative slopes correspond to the (usually expected) increasing steric exclusion from the gel matrix with increasing molecular size of the solute. Positive slopes, on the other hand, indicate that the partitioning favours the matrix with increasing solute size. Partial molar volumes have been used because it is considered to be the most suitable parameter to use in conjunction with the exclusion phenomenon and in describing the mutual interactions between components[2]. Partition coefficients are summarized in Table I.

Partitioning behaviour of solutes in a cellulose gel with different solvents

(a) *Polyethylene oxides* (*Fig. 1*). Differences in the partitioning of these compounds in water and DMF arise primarily because water is a better solvent than DMF for the epoxides; DMF is an aprotic solvent and the epoxides are proton

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TABLE I

PARTITION COEFFICIENTS FOR VARIOUS SOLUTES ON GELS OF CELLULOSE
AND ITS DERIVATIVES WITH DIMETHYL FORMAMIDE AS ELUENT

Gel	Partition coefficient, K_{av}*			
	Cellulose	Cellulose acetate	Methyl cellulose	Trimethylsilyl cellulose
*Cellodextrins***				
G-1	0.93_5	0.79_0	0.90_0	0.72_8
G-2	0.90_3	0.72_8	0.84_0	0.69_0
G-3	—	—	0.80_0	0.65_8
G-4	0.82_0	0.59_0	0.74_9	0.62_4
G-5	0.86_2	—	—	—
*Polyethylene oxides***				
$M = 190$	0.81_8	0.80_3	0.86_0	0.70_0
$M = 270$	0.80_0	0.78_2	0.85_0	—
$M = 400$	0.79_3	0.74_4	0.81_0	0.69_0
$M = 570$	0.74_5	0.68_6	—	0.67_4
$M = 990$	0.69_5	0.57_5	0.71_9	0.64_9
*Polyhydric alcohols***				
Ethylene glycol	0.92_0	0.86_9	0.80_6	0.70_0
Glycerol	0.94_5	0.84_0	0.85_9	0.71_6
Erythritol	0.97_0	0.82_8	0.91_2	0.73_4
Arabitol	0.99_1	0.80_7	0.90_6	0.72_0
Mannitol	1.02_5	0.79_0	0.89_5	0.70_9
*Cellodextrin acetates***				
G-1-Ac	0.80_5	0.81_9	0.76_3	—
G-2-Ac	0.76_4	0.70_2	0.67_2	0.61_3
G-3-Ac	—	—	0.59_4	0.60_7
G-4-Ac	0.70_2	0.62_5	—	0.59_8
*Methyl p-oligophenylenes****				
Toluene	0.79_0	Adsorbed		
2-Oligophenylene	0.78_0	0.81_5		
3-Oligophenylene	0.76_0	0.79_7		
4-Oligophenylene	0.73_2	0.76_5		
5-Oligophenylene	0.70_6	0.73_0		

* — $K_{av} = (V_e - V_0)/(V_T - V_0)$ where V_0 = void volume, V_T = total volume of swollen gel matrix and V_e = elution volume for a given solute.
** Solute loading, 14 mg.
*** Solute loading, 3.5 mg.

acceptors. The observed differences in partitioning are less than might be anticipated solely on these grounds, however, since DMF interacts more strongly with the cellulose hydroxyl groups than does water[12].

(b) *Polyhydric alcohols* $[CH_2OH(CHOH)_nCH_2OH]$ (*Fig. 2*). Partitioning is more complicated with these solutes because they interact more strongly with both the solvent and the gel matrix than is the case with the polyethylene oxides. In
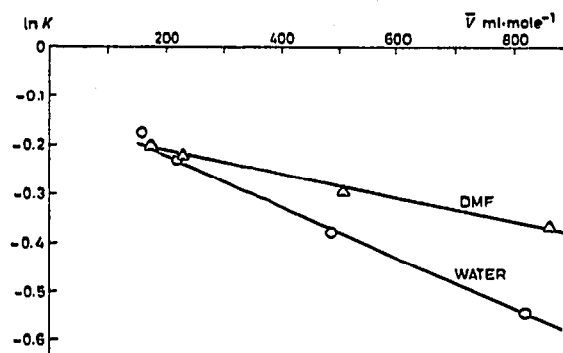

Fig. 1. Partitioning behaviour of polyethylene oxides on a cellulose gel with water and DMF as eluting solvents.
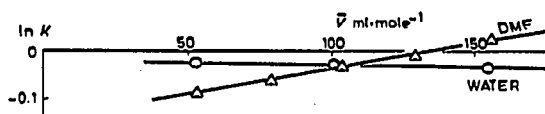
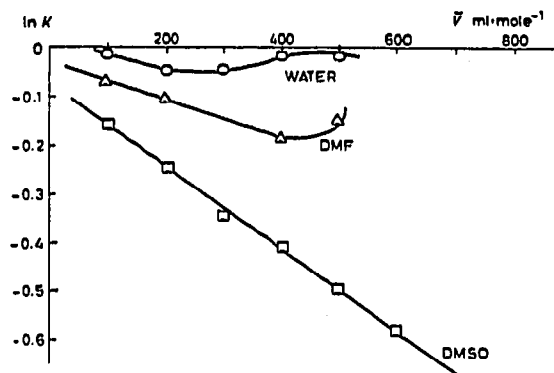Fig. 2. Partitioning behaviour of polyhydric alcohols on a cellulose gel with water and DMF as eluents.

Fig. 3. Gel chromatography results for cellodextrins on a cellulose gel with different solvents.

addition, DMF, which is more basic than the alcohols or water, interacts most favourably with the cellulose hydroxyl groups. The positive slope observed with this solvent is due to the superimposed effect of the decreasing solubilities of the alcohols in DMF with increasing molecular size. Intramolecular hydrogen bonding with these compounds[12] should be more pronounced in DMF, producing a more rapid decrease in solubility with increasing size than is found in water. In water, the solute–matrix and solute–solvent interactions are more closely matched, yielding only a slight molecular weight dependence.

(c) *Cellodextrins* (*Fig. 3*). Owing to their structural similarity, both the gel matrix and the solutes interact with the solvents according to the proton-accepting abilities of the latter: DMSO > DMF > water. Superimposed on this are solute–gel matrix interactions. With water as solvent, the following factors are relevant. The expected trend of increasing exclusion with increasing size is observed initially,

and thereafter solute interactions with the matrix become dominant, the sigmoid portion apparently typifying this system at temperatures less than 25°. Above this temperature, a monotonic increase in ln K_{av} with increasing size is observed[9]. This phenomenon presumably reflects changes in the character of cellulose as a function of temperature (cf., the observed inflection[8] in the specific volume of cellulose at 25°).

In DMF, the much lower solubility of cellopentaose causes the marked increase in ln K_{av} for this solute.

DMSO interacts strongly with both the solutes and the gel matrix, masking any tendency for solute–matrix interactions[5].

Partitioning behaviour of solutes in cellulose and derivative gels with DMF as solvent

(a) *Polyethylene oxides* (*Fig. 4*). The weakly polar proton-accepting epoxide group can interact only weakly with the cellulose hydroxyl groups and least favourably with the acidic acetate groups (Fig. 4a). Partitioning in the cellulose gel is also influenced by the competitive interaction of DMF with this matrix; for this reason, the partition coefficients are lower with the cellulose gel in comparison with methyl cellulose. The exclusion trend (negative slope) is most pronounced on the acetate gel because the substituent is acidic and the solvent is basic in the Lewis sense. A possibly greater steric exclusion, due to the size of the acetate groups, will be more than compensated for by the greater degree of swelling of the acetate gel. It will be noted that with the cellulose and acetate gels, the different swelling would be expected to produce a trend in partition coefficients opposite to that which is observed.
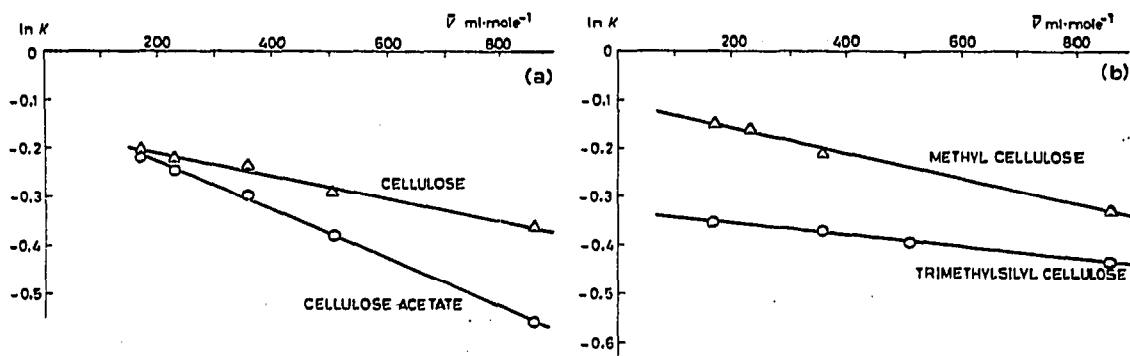

Fig. 4. Gel chromatography results for polyethylene oxides on (a) cellulose and cellulose acetate gels and (b) methyl cellulose and trimethylsilyl cellulose gels.

One would expect very similar interactions with both of the ether substituents, O-methyl and O-trimethylsilyl (Fig. 4b). However, the bulky O-trimethylsilyl groups substantially hinder accessibility to the matrix. Although qualitatively similar behaviour is observed for these gels (cf., Fig. 5), the partition coefficients for all solute types are consistently lower on trimethylsilyl cellulose. In general, it is observed that the partitioning of solutes on the methylated gel is intermediate between those on cellulose and acetate gels, which is the expected behaviour due to the slightly

basic character of the ether group in comparison with acetate (acidic) and alcohol (basic) groups.

(b) *Polyhydric alcohols* (*Fig. 5*). The partitioning trends in Fig. 5a can be explained by the relative abilities of hydroxyl and carbonyl oxygen atoms to function as proton acceptors. Clearly, the former are most favoured, as hydrogen bonding can occur, but as DMF is also a proton acceptor and interacts more favourably with the solute than the acetate gel, a negative slope results. A more complicated situation arises with the ether substituents O-methyl and O-trimethylsilyl (Fig. 5b). Here, both steric and energetic factors operate, with the solvent playing an important role. At the lower molecular weights, the alcohols can interact with the basic ether oxygen with little steric hindrance, giving an initial positive slope. At the higher molecular weights (above erythritol), steric factors hinder such interaction and the competing role of the solvent as proton acceptor is favoured. With erythritol, intramolecular hydrogen bonding leads to a double-ringed structure[12], whereas ethylene glycol and glycerol are single-ringed. This behaviour illustrates the interplay between these competing interactions. The bulky O-trimethylsilyl groups also hinder the access of solute to the matrix as discussed above.

(c) *Cellodextrins* (*Fig. 6*). These solutes can interact strongly with both the solvent and the cellulose matrix through hydrogen bonding. For this reason, interactions are most pronounced in this system (Fig. 6a) although the partition coeffi-
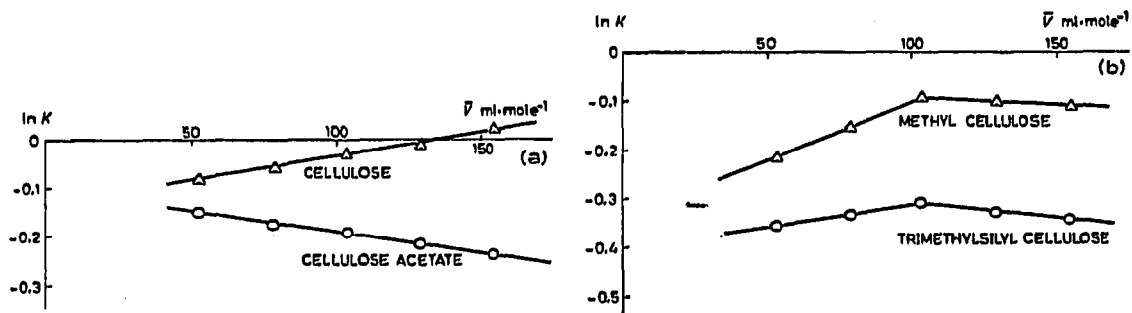

Fig. 5. Gel chromatography results for polyhydric alcohols [$CH_2OH(CHOH)_nCH_2OH$] on (a) cellulose and cellulose acetate gels and (b) methyl cellulose and trimethylsilyl cellulose gels.
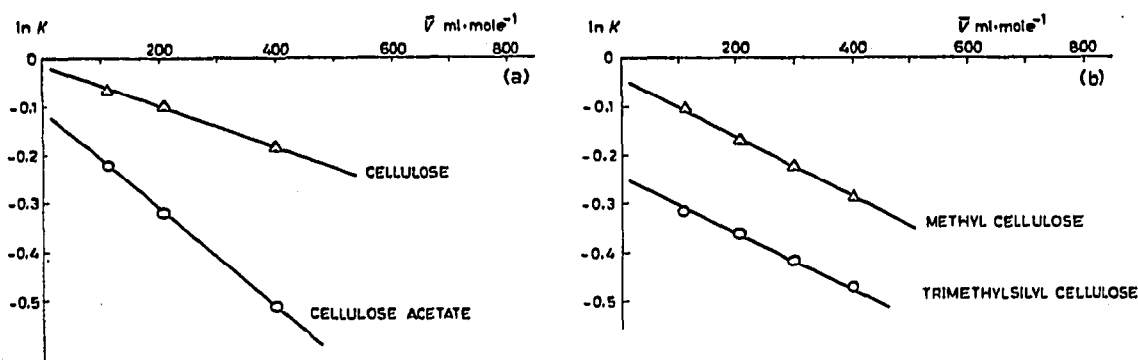

Fig. 6. Results for cellodextrins on (a) cellulose and cellulose acetate gels and (b) methyl cellulose and trimethylsilyl cellulose gels.

cients are lower than might be anticipated owing to the competing interaction of the solvent with the cellulose hydroxyl groups. With the acidic acetate groups, partitioning favours the solvent more, owing to its greater tendency to accept a proton from the solute. The behaviour of these solutes with the ether substituents (Fig. 6b) is intermediate, reflecting the slightly basic character of these functional groups. Interaction with the matrix, as observed for the alcohol series, will be hindered by steric factors with these more bulky solutes. Furthermore, the lower partition coefficients for the lower members of the series on the trimethylsilyl cellulose gel (compared with the cellulose acetate gel) show that accessibility of the solute to the matrix itself, as distinct from the accessibility of the ether oxygen atoms, is also more impeded. The greater negative slopes in these plots in comparison with those for the polyethylene oxides (Fig. 4) illustrate the more pronounced solute–solvent interactions with the cellodextrins.

(d) *Methyl-p-oligophenylenes* (*Fig. 7*). It can be seen that, in constrast to the results obtained with weakly polar solutes, the partition coefficients are higher for the cellulose acetate than for the cellulose gel. An analogous but more pronounced reversal in partitioning has been observed with polar solutes on a polystyrene gel[1]. In the present instance this effect is due to the favoured interaction between the basic methyl p-oligophenylenes and the acidic acetate groups. It will be noted that the first member of the series, toluene, interacts strongly and irreversibly with the
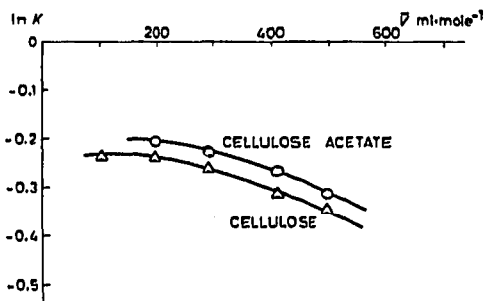

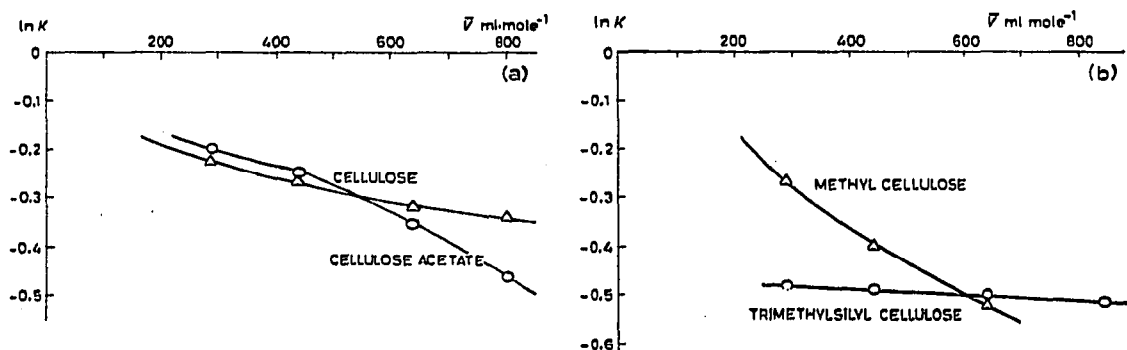Fig. 7. Results for methyl p-oligophenylenes on cellulose and cellulose acetate gels.

Fig. 8. Results for cellodextrin acetates on (a) cellulose and cellulose acetate gels and (b) methyl cellulose and trimethylsilyl celullose gels.

acetylated gel. DMF also interacts preferentially with the cellulose gel, contributing to the lower partition coefficients with this matrix.

(e) *Cellodextrin acetates* (*Fig. 8*). These solutes interact very weakly with the cellulose gel but more favourably with the acetate-substituted gel (Fig. 8a). These remarks regarding the solute–matrix interactions are based on temperature-dependence studies in these systems[9]. DMF can also interact favourably with the cellulose matrix, giving lower partition coefficients than would otherwise have been the case, *i.e.*, the curve for the cellulose gel is displaced downwards while that for the acetate gel is displaced upwards by the solvent effect. The opposite curvatures apparent at the higher molecular weights reflect increasing exclusion from the acetate gel (energetic interactions are less important). With the cellulose gel, on the other hand, partitioning increasingly favours the matrix.

Fig. 8b illustrates the results with ether groups on the gel. In these systems, there are possibly weak interactions between the acidic solute and the slightly basic ether substituents of the gel but stronger interactions between these more polar gels and the solvent. With trimethylsilyl substitution, the essentially similar interactions are almost completely masked by the very limited accessibility to these bulky solutes of the gel matrix; thus there is very little resolution as a function of molecular size.

It can be concluded that partitioning behaviour is a complex pattern depending on both steric and energetic interactions between solute and gel. Nevertheless, it has been possible to identify positively certain features of the interactions determined by the functional groups present in the solute and gel matrix. This is best exemplified by the behaviour of the polyhydric alcohols with respect to hydroxyl, ether and ester groups, depicted in Fig. 5.

Important factors are:

(a) the competing interactions of solvent with the gel and solute;
(b) the steric impediments imposed by substituents in the vicinity of the reactive centre of the functional group;
(c) the steric influence of the bulk of the functional group as a whole on the access of the solute to the gel matrix.

TABLE II

INCREMENTS IN ln K RELATIVE TO CELLULOSE FOR VARIOUS SOLUTES AND GELS

$\Delta \ln K = (K_{\text{cell-ox}} - {\text{cell-OH}})$.

Gel	Solute			
	Polyethylene oxide 200	Glucose acetate	Glucose	Mannitol
Methyl cellulose	+0.050	−0.048	−0.037	−0.135
Cellulose acetate	−0.016	+0.022	−0.155	−0.260
Trimethylsilyl cellulose	−0.155	−0.258	−0.255	−0.368

Leo *et al.*[10] have discussed partitioning in terms of the functional groups of the solute. In an analogous manner the quantity

$$\Delta \ln K = [\ln K_{cell\text{-}ox} - \ln K_{cell\text{-}OH}]$$

can be used to describe the shift in the partition coefficient for a particular solute in a derivative gel (cell-ox) relative to the cellulose gel (cell-OH). Table II lists $\Delta \ln K$ for solutes of similar molecular weight: mannitol ($M = 182$), glucose ($M = 180$), polyethylene oxide 200 ($M = 188$) and glucose acetate.

Fig. 9 depicts the data. The position on the vertical axis gives the $\ln K_{cell\text{-}OH}$ value for the compound and the horizontal displacement gives the corresponding $\Delta \ln K$ value on each gel relative to cellulose. The over-all symmetry of the plot is noteworthy.
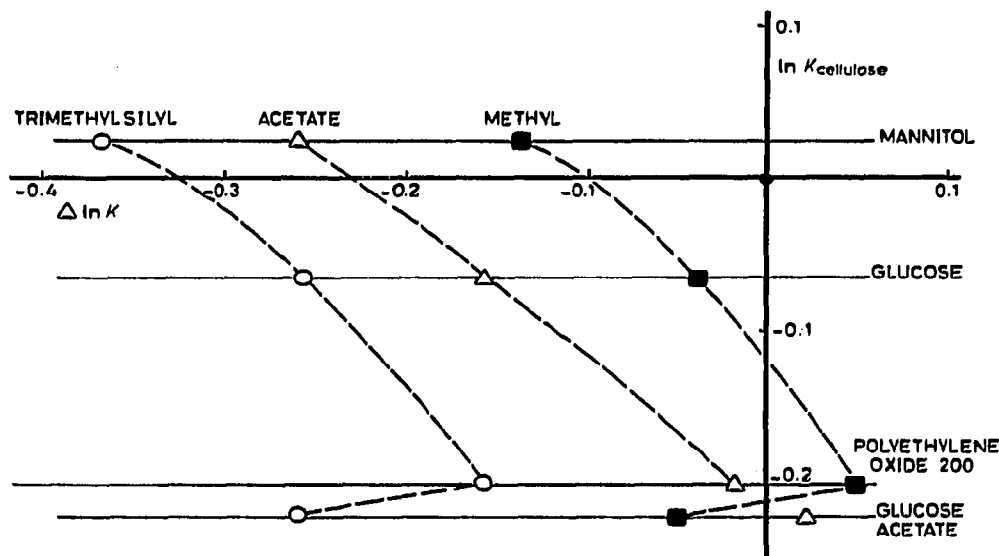

Fig. 9. Increments in ln K relative to cellulose for various solutes and gels. The position on the vertical axis gives the ln $K_{cellulose}$ value for each compound and the horizontal displacement the shift in ln K on each gel relative to the value on the cellulose gel. The larger is $\Delta \ln K$, the greater is the exclusion from the gel phase.

There is an inverse relationship between the degree of interaction of a given compound with the cellulose gel and its interaction with a particular derivative gel. Thus, for example, the most polar compound, mannitol, interacts strongly with the cellulose gel and weakly with the methyl gel, while the least polar polyethylene oxide interacts weakly with the cellulose gel but strongly with the methyl gel. Furthermore, for the three polar compounds, the increment in ln K between two derivative gels is approximately constant. The effective polarities of the substituents are therefore in the order: methyl > acetate > trimethylsilyl. Also, steric exclusion due to the relative bulk of the substituent groups should lie in this sequence. Glucose acetate diverges markedly owing to the bulk associated with the five pendant acetate groups. The point for this compound on the acetate gel curve departs from the over-all pattern, revealing a pronounced acetate–acetate interaction.

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