INCLUSION PHENOMENA IN A SYSTEM DESIGNED FOR EXCLUSION - A CAVITARY PROBLEM AND A MODEL

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ABSTRACT. The Sephadex gels were introduced for their molecular-sieve-like properties. For some types of solute, however, they exhibit affinity for which the presence of water seems mandatory. Partitioning in these gels has been attributed to steric exclusion, hydrophobic interactions, dispersion interactions, etc. but may be due to a common structural feature , possibly a cavitary structure. Structural and partitioning similarities between the Sephadex gels and the cycloamyloses support this concept. A simple cavitary model vicinal water shell (CVS) is proposed in which the selectivity of the gel depends essentially on internal partitioning between a two phase system consisting of a vicinal-matrix-perturbed water shell lining the cavity and a core of normal (bulk) water. The CVS model cannot be used as yet to predict K_d values from the physico-chemical properties of the gels alone. The starting point is a known K_d value in one gel and the K_d value of the same solute in another gel is then calculated using the physical-chemical properties. The model was tested on several solutes and yielded promising results. Its implications are discussed.

1. INTRODUCTION

The Sephadex gels are covalently cross-linked but otherwise unsubstituted dextrans (6-O- α -linked polyglucans with limited branching) and were introduced about a quarter of a century ago with the aim of providing molecular sieves for use in chromatographic separations on a size basis (1,2). These ubiquitous gels have subsequently proved exceedingly useful for a multitude of applications. The molecular size range over which a particular gel is an effective sieve varies with the water content of the fully-swollen state as shown in Table 1.

Almost from the first day they appeared it has been known that in addition to their molecular sieve properties they have affinity for certain classes of solute, e.g. non-polar or weakly polar saturated compounds (3), certain aromatics (4), etc.

It is evident that there are several partitioning mechanisms e.g., for non-electrolytes steric exclusion, hydrophobic interactions and Van der Waals-

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London dispersion interactions, and in addition for electrolytes ion-dipole interactions (5). If the partitioning solute also contains a group capable of hydrogen bonding, stable gel-solute hydrogen bonds are unlikely in the presence of a large molar excess of water (6). Recently, however, a pattern in the partitioning behaviour among different gels was noted (7) which led to the proposal that despite the multiplicity of partitioning mechanisms a common structural feature of the gel might underlie them all (8). It might thus be possible to explain all, or at least, most of partitioning, i.e. steric exclusion and interactions, in terms of a simple unifying model. We have attempted to fulfil this aim. However, as our knowledge of the gel is not sufficient to allow predictions of solute K_d values from the physico-chemical properties alone, we have started out from a K_d value in one gel and the proposed model has then been used to predict the K_d values of the same solute in other Sephadex gels.

TABLE 1
PROPERTIES OF SEPHADEX GELS

Type	Nominal water regain[a] kg / kg	Exclusion limits kDa
G-200[b]	20	$4 \cdot 10^2$
G-100[b]	10	10^2
G-50	5	7-10
G-25	2.5	3-4
G-15	1.5	1.5
G-10	1.0	1.0

[a] the amount of water contained in fully swollen gel beads.

[b] calibrated with globular proteins; the other gels are calibrated with dextrans

2. THE RELATION BETWEEN SOLUTE STRUCTURE AND PARTITIONING

There are several different Sephadex gels available, varying considerably in water content and molecular sieving range. As will be evident from the following discussion the partitioning properties of the various gels are only quantitatively and not qualitatively different. We have therefore selected the highly cross-linked type Sephadex G-15 (Table I) as a starting point for our discussion.

As Fig 1 shows there are two archetypes of partitioning behaviour (9). For

polar solutes such as oligosaccharides, polyols and poly(ethylene oxide)s the logarithm of the distribution coefficient (ln K_d) decreases linearly with increasing molecular weight or degree of polymerisation (DP). The positive linear free energy relation (LFER) is characteristic of partitioning dominated by molecular sieving or steric exclusion.

In the case of non-polar or weakly polar saturated compounds the behavioural pattern is quite different. The ln K_d values of these solutes increase with increasing molecular size. At least for the 1-alkanols the increase of ln K_d is not, however, linear with respect to the number of carbon atoms (n_C) in the alcohol but instead there is a quadratic relationship (10) with a LFER with respect to the square of n_C, as has also been reported for linear 1, ω-diols (7).

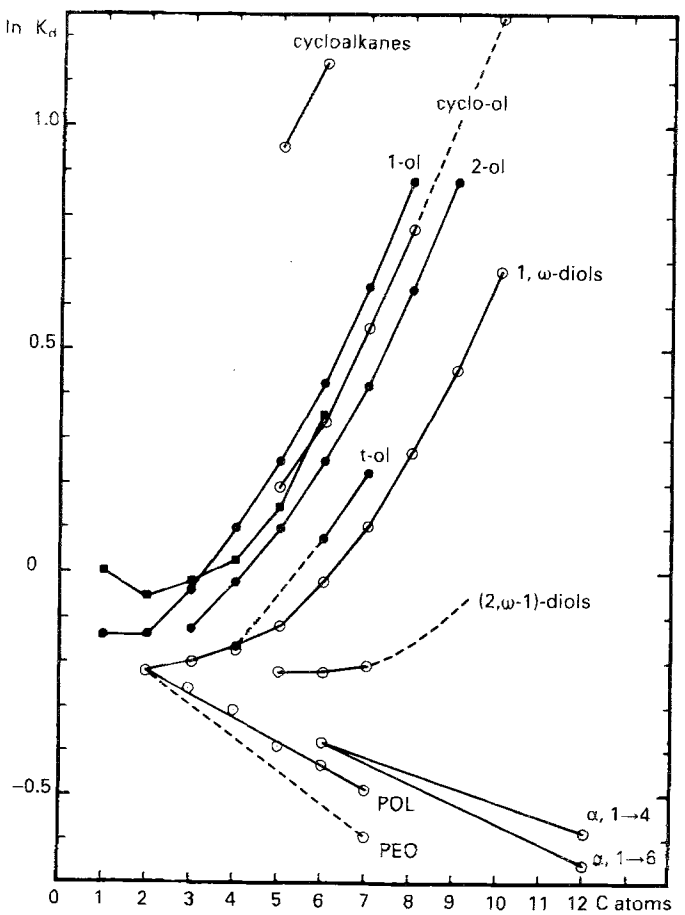

Figure 1. Distribution coefficients of saturated compounds in Sephadex G-15 at 25°C. Notation: 1-ol etc. refer to aliphatic alcohols, POL = polyhydric alcohols, PEO = poly(ethylene oxide)s and α,1 - 4 and α,1 - 6 are the slopes of the malto-and isomaltodextrin series respectively.

The affinity type of partitioning behaviour is not confined to non-polar or weakly polar solutes, but is also shared by aromatics, both polar and non-polar, and by non-aromatic Π-electron containing compounds such as amides and urea and thiourea and some of their derivatives.

For non-polar and weakly polar compounds the main, but not the only, determinant of partitioning, seems to be a hydrophobic interaction (HI) (9-12). The changes in the values of the thermodynamic transfer functions in ascending a homologous series are consistent with this notion. Further, the affinity for this type of solute most probably requires the presence of water and K_d values are affected by the addition of water-structure perturbants (13). There is also intermediate behaviour in molecules belonging to a series in which the polarity changes with carbon chain length; an example is shown in Fig 1 where the ln K_d values of the 2,(ω-1)-diols do not change appreciably among the lowest members, whereas at greater carbon chain lengths the series would behave like the monohydric alcohols.

3. THE GEL STRUCTURE AND SOLUTE PARTITIONING

There is a distinct difference between the partitioning patterns of polar and non-polar solutes among the different Sephadex gels as illustrated in Fig 2. Thus the polar solute is more excluded, the lower the water content, i.e. the K_d value of a polar solute is lower the more highly cross-linked the gel. The opposite holds for a non-polar compound, the greater the gel matrix concentration, the higher the affinity. Thus,in some way a high matrix concentration and a higher degree of cross-linking cause the gel to behave more hydrophobically, i.e. the affinity for non-polar molecules is increased.

The major building units of the Sephadex gels, the anhydroglucose residues (AGR), have non-polar regions on the faces of their pyranose rings in their preferred chair ($^4C_1$) conformations. There is evidence that these regions may engage in (HI) (14,15). They thus constitute potential sorption sites for non-polar solutes, although Yano and Janado (11) suggested that some cooperativity would be necessary since the AGRs alone seemed insufficient to render the gels sufficiently hydrophobic unless the dextran chains were so arranged as to constitute a stable and regular array of their hydrophobic faces. As discussed below the present state of our knowledge of the structure of these gels indicates that some sort of array may exist. However, it must also be noted that the AGR are by no means the only potential sorption sites. Thus, the cross-link unit structures (CLU), i.e. mono- or diether-linked glycerol also constitute an appreciable fraction of the matrix in the most highly cross-linked gels. In the latter gels there is also evidence from NMR studies (17,18) that the mobility of the dextran chains is greatly reduced, a state, which should tend to produce a stable array of AGRs which together with their increased concentration might be expected to promote hydrophobicity, especially in view of the higher degree of substitution of the AGR hydroxyl groups.

The CLU also modify the gel structure, making it less "sugar-like". A detailed fragmental analysis (19) of a more highly cross-linked Sephadex gel (G-25 -see Table I) revealed that more than a third of the CLU were present as mono ether linked pendent side-arms and of the rest most were coupled to two ether

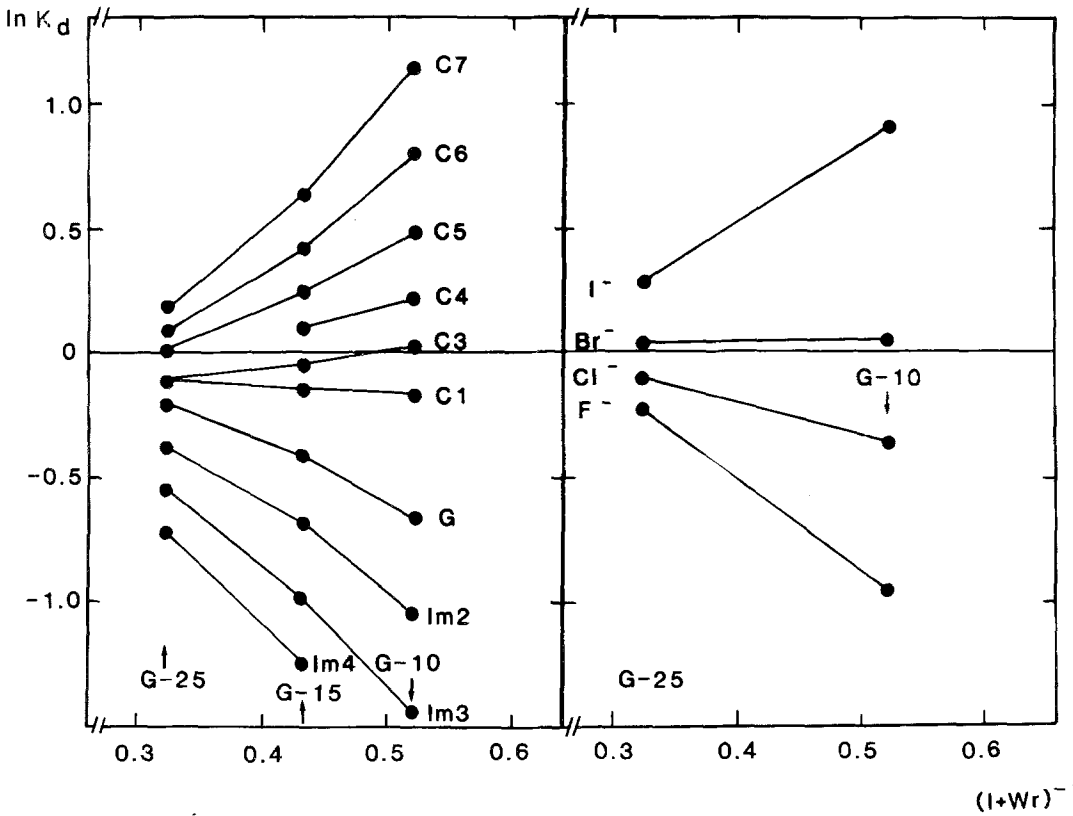

Figure 2. Partitioning behaviour in different gels. The abscissa is the matrix concentration (kg/kg of water-swollen gel beads). The matrix concentration corresponding to the different gels are indicated by arrows. The left-hand part shows the non-electrolytes. C1 - C8 are the 1-alkanols, G = glucose; Im 2,Im 3 and Im 4 are the biose, triose and tetraose respectively of the isomaltose series. The right-hand part gives the halide values.

oxygens on the same AGR forming 1,4-dioxane rings (alongside the AGR). Only a few per cent of the CLU formed true cross links, thus contributing to the gelling process. The degree of cross-linking is thus much lower than was once thought but this new concept is confirmed by recent NMR studies (18). Thus, a highly cross-linked Sephadex gel can be regarded as consisting of covalently cross-linked dextran chains, heavily modified by the presence of covalently bound CLU and therefore the changes in the composition, as well as in the concentration of the matrix should be taken into account when calculating K_d values in the CVS model (see below).

It is also probable that the matrix surface is more densely arranged in some localities due to the necessity for non-random packing. Thus, calculations of the mean centre-to-centre distance of the dextran chains revealed the impossibility of a purely random network in the most highly cross-linked gels, since in this case the mean inter-chain distance would be less than their Van der Waals diameter (in Sephadex G-25 and the more highly cross linked members G-15 and G-10).

Theoretically the polymerisation (cross-linking) process should not result in a random network of dextran chains but in a heterogeneous gel with cross-link-dense, matrix-rich, water-poor domains intermingled with cross-link-sparse, matrix-poor, water-rich regions (2,20).

Electron microscope studies on other gels such as agarose (21) and polyacrylamide (22,23) have revealed no indication of a random network; instead there was a more open microcellular structure and it was concluded that the microcells were bounded by sheets, or in agarose by filaments, of closely apposed polymer chains. Assuming that Sephadex gels have a similar structure, the overall picture of the gel is of cavities bounded by filaments or sheets formed of modified chains containing both substituted AGR and 1,4-dioxane rings and pendent glyceryl side-arms. The changes in partitioning behaviour which occur as a result of increased cross-linking (Fig 2) indicate an increased hydrophobicity as evidenced by the behaviour of the 1-alkanols. Since the opposite type of behaviour, so called steric-exclusion, i.e. decrease in K_d value with increased matrix concentration is characteristic of polar saturated comounds, it might equally well be referred to as decreasing hydrophilicity. These effects resulting from increased cross-linking raise the intriguing question of where the different types of solute are accomodated.

The Flory polymerisation theory (20) leads to a heterogeneous picture of the gel and a distinction between the matrix-dense, water-poor domains and the matrix-sparse, water-rich regions seems well-nigh tailor-made as a two-site system for achieving a spatial separation of non-polar and polar solutes with preferential sites for both. Further, qualitatively at least it would provide an explanation for the different partitioning patterns of the two types of compounds in different Sephadex gels. Thus, as the degree of cross-linking increases, the matrix-rich water-poor domains will expand at the expense of the water-rich spaces, which will become smaller; thus the K_d values of non-polar solutes will increase while those of the sterically excluded polar solutes will be lower.

This model in fact provides three sites in that a transition region between the two domains may also be of significance in the partitioning, for example, of a molecule with both polar and non-polar groups.

As shown in Fig 3 there is a generalized relation between all the Sephadex gels studied (7). In this figure the ln K_d values of different solutes in each gel are plotted against those in Sephadex G-15. These ln K_d plots include a wide spectrum of solute types, ranging from polar oligosacharides, polyols and poly(ethylene oxide)s to aliphatic and aromatic hydrocarbons, alkanols, phenols and monohalo benzenes. In the case of each gel a straight line with a high linear correlation coefficient ($r^2 > 0.985$) was obtained and further, each of the lines intersected at nearly the same ln K_d value (approximately zero). This indicates that the different Sephadex gels behave proportionately with respect to one another. The slope of the line is thus a normalized logarithmic distribution coefficient (NLK). This suggests that, at least in Sephadex gels, either all the different solutes have a common partitioning mechanism which seems unlikely and/or that all solute partitioning depends on a common structural feature of the gel.

In the case of polar solutes steric exclusion seems to be the dominant partitioning mechanism and it is well documented that this can be attributed to the presence of solvent(water)-filled pores or cavities (24,25). A cavitary system may thus be the structural basis for partitioning in general thus

involving the apparent paradox that steric exclusion belongs to the class of inclusion phenomena.

The problem therefore is to explain how cavities can subserve at least two functions, i.e. firstly as accumulation sites for solutes exhibiting affinity and secondly as pores restricting the entry of certain types of solute on a size basis, although the latter mechanism may be, as mentioned above, a question not only of size but also of polarity.

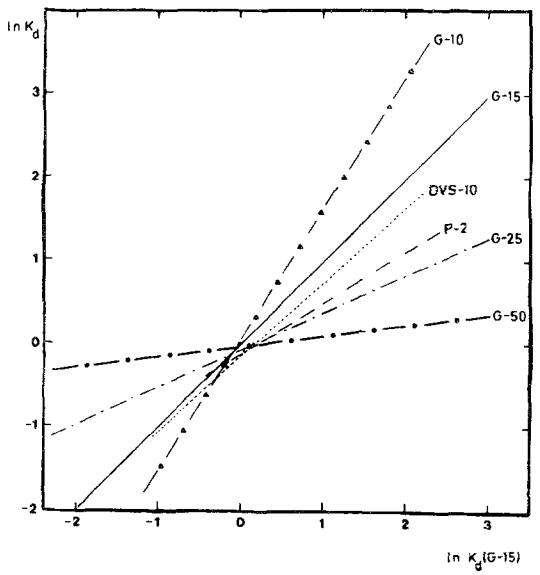

Figure 3. Values of ln K_d in different gels at 25^0 C plotted against ln K_d values in Sephadex G-15. See text for discussion. The G-numbers refer to Sephadex gels, DVS-10 is a divinyl sulphone cross-linked dextran gel (water regain = 0.99) and P-2 is polyacrylamide Biogel P-2 (water regain = 1.8).

4. CYCLOAMYLOSES AS PARADIGMATIC CAVITARY MODELS FOR SOLUTE PARTITIONING IN SEPHADEX GELS

In being hydrophilic, in the sense that they swell in water, and exhibiting affinity for non-polar solutes the Sephadex gels are by no means unique (5) and in this respect the Schardinger cycloamyloses (CA) appear to be potentially very useful models (26). Not only do the CA exhibit similar partitioning properties but they are molecules whose cavitary surface bears a striking structural resemblance to that of Sephadex gels. The interior of the CA cavity is not hydrocarbon-like (27,28) nor, as is sometimes claimed, non-polar (hydrophobic), although it can be regarded as relatively hydrophobic when compared with water (29). When the CA are dissolved in water their partial specific volumes (30) are not significantly different from those of maltoheptaose or D-glucose indicating that the cavities are not empty but occupied by water molecules. A cavitary structure seems necessary for complex formation since, whereas the solubilities of organic acids were increased in the presence of CA, they were not increased by glucose, α-methylglucoside or maltose (31).

The cavity of the CA in fact, contains a region of high electron density (32) and may behave as a Lewis base (33) . The glycosidic oxygens are exposed centrally in the cavity forming a ring and each is surrounded by a square array of four methine groups (C3 and C5) from adjacent AGRs. Further, the C6 hydroxyls lie on the edge at the narrow end and the C2 and C3 hydroxyls at the broad end of the torus (34).

There is also spectroscopic evidence about the properties of the interior of CA . Thus the spectrum of 4-tert-butylphenol complexed with cyclohexaamylose (C6A) in aqueous solution was superimposable on that of the phenol in dioxane (29), thus indicating the dioxane-like interior of the C6A cavity. Further, the difference spectrum between an aqueous mixture of C7A and N-acetyl tyrosine ethyl ester (ATEe) and ATEe in water alone was essentially identical to that between ATEe in ethyleneglycol and ATEe in water (35), which also underlines the similarity between the CA cavity and the structure of Sephadex.

It is probable that HI play a role in stabilising adduct formation with CA. There has been some divergence of opinion on this point that seems to have been due to two reasons, firstly the failure the take into account the possibility that HI might not be the only, or even the major, interaction and secondly, it has been sometimes assumed that the aromatic ring is non-polar. That this is a fallacy is evident in data from Sephadex G-15. In this gel the C6 cyclic hydrocarbons, cyclohexane, cyclohexene, the cyclohexadienes and benzene have approximately similar distribution coefficients. The ΔH^{o} values for transfer into the gel, however, decrease by about 2 kJ/mol for each double bond (9). This implies that in this system benzene is less hydrophobic than cyclohexane by 6 kJ/mol. If the partitioning of compounds such as the 1-alkanols is considered it is evident that both a hydrophobic interaction and an energetic interaction are involved in complexation with CA (36,37).The latter interaction seems to increase in magnitude the tighter the fit of the alcohol in the cavity and a London-Van der Waals dispersion interaction probably accounts for this (36).

Although, as might be expected in view of their small well-defined cavities, the CA exhibit considerably more affinity than the gels, there is a striking parallelism between the affinities of solutes for the two systems. This, combined with the similarity in internal surfaces would seem to support the notion that in Sephadex also a cavitary structure may be important.

The normalized ln K_d value (NLK-see Fig 3) and proportionality between different gels suggests that the partitioning of all types of solutes has some common feature. In fact, this relation also holds, albeit a little less precisely, for two other types of gel, in that straight lines were obtained which intersected near to, but not quite within, the very restricted area containing the Sephadex gel intersects. The other two gels studied were a closely related divinyl sulphone cross-linked dextran and the unrelated type polyacrylamide, P-2.The fact that the latter conforms so well to the behaviour of the others suggests that the partitioning properties do not depend on a specific chemical structure of the gel.

The NLK does not, of course, exclude a two site model, although it places rather heavy demands on it, in that between any two gels the differences in each type of site must be such that the same degree of proportionality is maintained.

5. THE CAVITY VICINAL WATER SHELL (CVS) MODEL

It is not necessary to define the properties of the cavity wall at all, except as regards its ability to perturb the structure of water in its immediate vicinity, the nature of the structure of the vicinal water is thus totally disregarded. The thickness of this vicinal water layer (38,39) will be assumed to be 0.8 nm, i.e. the distance over which water structure is apparently ordered as reported from X-ray diffraction studies on liquid water (40).

In addition, the presence of normal bulk water in the central part of the cavity requires a certain minimal volume (39). The minimal number of water molecules required has been assessed as between 10 and 10,000 , corresponding to a spherical volume between 0.4 and 4 nm in radius, with the real value much nearer the lower limit. In the model described here only a vicinal water layer 0.8 nm thick will, however, be taken into account but it must be borne in mind that this is probably an unwarranted oversimplification. As regards the partitioning of non-polar solutes the model is in essence a simplified adaptation of that postulated by Tabushi et al. (41) to account for the driving forces for inclusion in C6A (see also Refs. 3,11,42).The entropy changes associated with the transfer of a non-polar group from bulk water to the water inside the gel cavities may be treated as occurring essentially in two stages, which involve an entropic dissymmetry, due to the different states of water in the two locations. Only the entropy changes due to water-water interactions arising from the presence of a non-polar group will be considered here (43,44).

Firstly, the collapse of the water-cage vacated by the transferred group yields an entropy increase and if (allowing for other entropy changes) this is not balanced with a corresponding entropy decrease when the group is located in the cavity then the latter will become a preferred site. Within the vicinal water of the cavity the situation is different from outside. The effect on the water structure, of a group which is electrostatically neutral, is due mainly to its lack of influence on water-water interactions. As the vicinal water is influenced by the matrix, the non-polar group should have little, if any, effect on its structure, thus yielding a net entropy increase and a preference for the cavity.

It is not, however, only non-polar solutes which apparently prefer the vicinal water. Thus, polar Π-electron containing compounds, such as urea and thiourea and aromatic compounds also exhibit affinity for the gels (9). The vicinal water is also most likely accessible to certain ions, i.e. highly polarisable species such as I^-, SCN^- and ClO_4^- which have K_d values well in excess of unity (16,45).

The halides present an interesting case (right hand side of Fig 2) in that the differences between their K_d values in the two Sephadex gels G-25 and G-10 exhibit divergent trends. Thus, I^- exhibits affinity, the K_d values exceed unity in both gels and there is a higher value in G-10, which has the higher concentration of matrix. Cl^- and more markedly F^-, on the other hand, behave in a way similar to polar saturated compounds such as oligosaccharides whose partitioning is dominated by steric exclusion; their K_d values are lower in the more highly cross-linked gel, i.e. in the gel with smaller pores(46). Now it is most unlikely that these gels exclude F^- more than I^- for steric reasons. It is true that the hydrated radius of F^- is a little greater (47), but G-10 has an exclusion limit of several hundred daltons. This conclusion is further greatly strengthened by the marked dependence of anionic partitioning on the nature of the eluent

anion. Thus, whereas changing the latter may greatly change all test anion K_d values, it does not affect their order (46).

It is therefore much more likely that the gel, or at least, part of it, is less readily accessible, for other than steric reasons, to F^- than I^-. This is more pronounced in the gel G-10 which, having the higher matrix concentration, also must have a higher fraction of its water vicinal.

The possible accumulation of I^- in the vicinal water has a possible analogy in the observation made long ago (48,49) that the order of exclusion, in favour of bulk water, from another vicinal situation, the air-water interface, was as in Sephadex, $F^- > Cl^- > Br^- > I^-$. If this is a valid analogy it seems that we might seek the mechanism of the halide selectivity in their interaction with vicinal water. Thus, the highly polarisable, poorly hydrated I^- must be most easily accomodated there and the most hydrated F^- (50) least. This may result from differences in the compatibility of the anion hydration shells with the vicinal water (51) or possibly be a question of solubility differences (52).

6. USE OF THE MODEL IN PREDICTING PARTITIONING

There is as yet, insufficient information about the structure of the gels to attempt to predict the K_d value of a solute without using partitioning data from other solutes or another gel. What we attempt to do here is to predict the halide and some non-electrolyte K_d values in other gels using the K_d values in the gel G-10 as a starting point.

The Cavity-vicinal water shell (CVS) model is shown diagramatically in Fig 4. It consists of a spherical cavity lined with a shell of vicinal water (p) enclosing a core of normal, unperturbed water (u). The real cavity in the gel

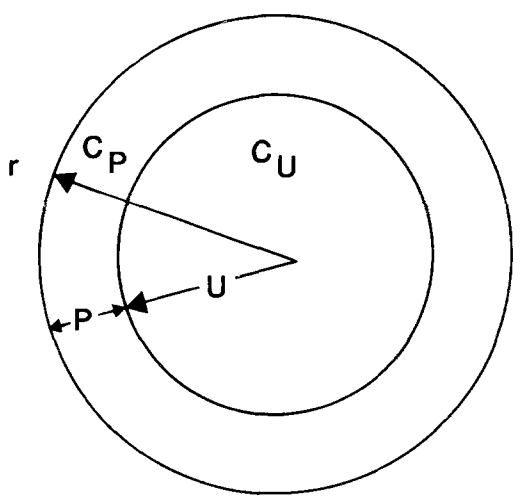

Figure 4.The cavity-vicinal water shell (CVS) model. See text.

will of course be an interstice of undefined shape between the matrix chains. The predicted K_d values (crosses) in Fig 5 have been calculated assuming r = 2.0 nm and p = 0.8 nm.

Starting from the K_d values in G-10 the distribution coefficient within the cavity (C_p/C_u) is first calculated. From this we then calculate the change in K_d in another gel due to the different water regain value in the latter, the change in cavity volume being assumed to be proportional to this. The new K_d value so obtained is then multiplied by weighting factors to take into account the changes in both matrix area and content of ether oxygens as there is a correlation between the latter and the K_d value (7,53). The last mentioned data are obtained from structural studies on these gels(19,53).

The results in Fig 5 show that the K_d values in G-25 were predicted accurately for Cl^-,Br^- and I^- (the result for Br^- is not significant as K_d does not differ much from unity); for F^-, however, there was a small discrepancy.

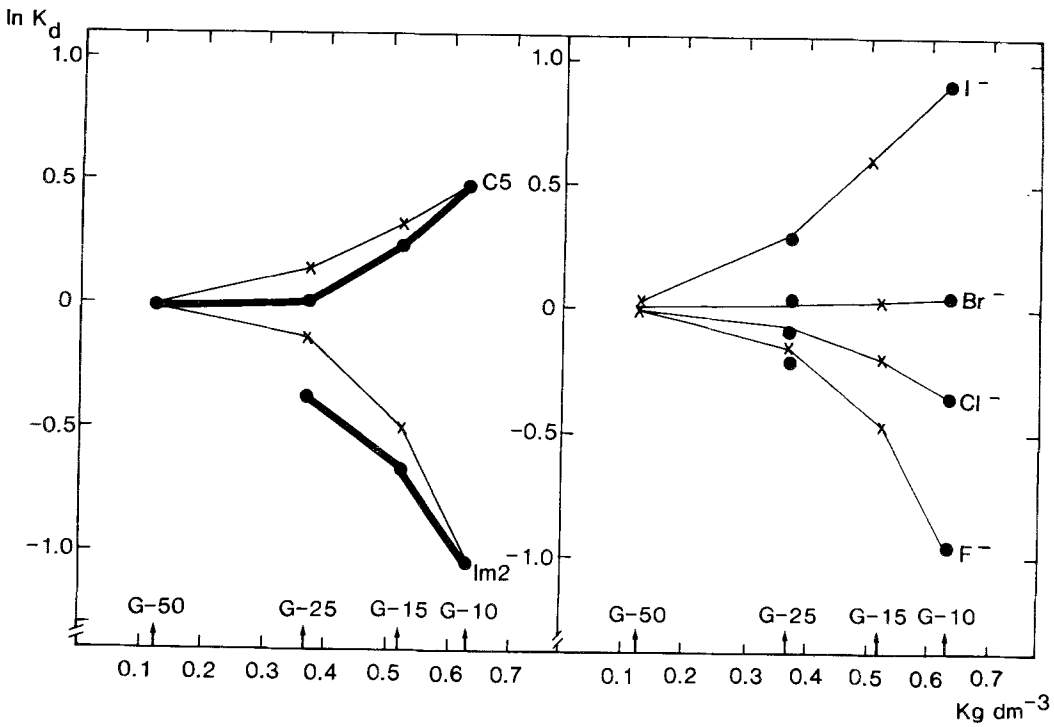

Figure 5. Partitioning data from Fig 2 and predictions of the CVS-model made from the K_d values in Sephadex G-10. The crosses joined by thin lines are the predicted values and filled circles are observed values. The predicted values for F^-, Cl^- and Br^- in Sephadex G-25 have been omitted because of overlap with the observed values. C_5 is 1-pentanol and Im2 is isomaltose.
Note. The matrix concentration is expressed in kg/dm^3 water-swollen gel beads.

At least as far as the halides are concerned the model is internally consistent and, considering the gross over-simplification and approximations involved, appears to be a promising approach and will it is hoped stimulate further structural studies of these gels. In the case of the non-electrolytes, 1-pentanol, 1-hexanol (data not shown) and isomaltose, although the predicted values exhibited the same trend as the observed values, the fit was not so good as with the halides (Fig 5). This is perhaps not so unexpected as these non-electrolytes are much more complicated structures than simple ions . Further, in the quasi-neutral gel anion behaviour is not complicated by ion exchange.

Finally, there is one more problem as regards steric exclusion. Since the radius of the cavity will represent a maximal size for a penetrating solute, the ln K_d values for a series such as the 1-alkanols which exhibit affinity (see Fig 1) for the gel cannot rise indefinitely, but must be expected to fall as the effective solute radius approaches and exceeds that of the cavities. The data shown in the figure do not illustrate this problem, since measurements on 1-alkanols of a size near to the exclusion limits found for polar molecules are impossible due to their far too low solubilities in water. In view, however, of possible energy differences required for encroachment of a solute into the layer of vicinal water, the latter may effectively reduce the size of the space available to an entering solute. Thus, the concept of the exclusion limit for polar solutes, such as oligo-saccharides becomes diffuse if the boundaries of the largest spaces they can enter are to be defined in terms of a phase separation (54) between the vicinal water lining the cavity and the normal (bulk) core water. The real situation may be more complicated since, if the cavities are small, the entering solute by virtue of its size may profoundly disturb the original "stratification" of the water in the cavity .

However, if this notion is correct, molecules belonging to the two archetypes of partitioning behaviour shown in Fig 1 may have different exclusion limits in the gel. This could be verified by showing that if a given oligosaccharide was completely excluded, an even larger molecule of the type with affinity for the gel was not, but was instead distributed throughout the whole gel bead. This experiment will be easy to perform if there is a large discrepancy in the exclusion limits of the solutes belonging to the two categories, otherwise it may be difficult to obtain an unequivocal result because of the problem of assessing which relevant size parameter is to be used.

A puzzling problem in the behaviour of the highly cross-linked Sephadex gels has been the order of K_d values of three aldohexoses, i.e. galactose <glucose < mannose (3). These sugars, all in the $^4C_1$ conformation have local non-polar domains (14,15) and Miyajima (55) has recently calculated that the order of increasing hydrophobicity is just that of the K_d values. In terms of the vicinal water model the K_d values of glucose and mannose would suggest an increasing degree of compatibility with the vicinal water.

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