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THE PARTITIONING OF 1-ALKANOLS AND OTHER COMPOUNDS IN SEPHADEX G-10 WHEN ELUTED WITH FORMAMIDE OR AQUEOUS SOLUTIONS OF UREA, GUANIDINIUM SALTS AND SOME SIMPLE ELECTROLYTES

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SUMMARY

Substitution of formamide for water as eluent greatly reduced, if not abolished, the affinity of Sephadex G-10 gel for higher 1-alkanols. Aqueous urea and guanidinium chloride (GuCl) also reduced the affinity although to a lesser extent than pure formamide. The reduction in affinity was greater for higher alkanols, so that in formamide the latter behaved as though steric exclusion dominated their partitioning. Not only was the affinity reduced but also the standard enthalpies and entropies of transfer; in water these become increasingly positive as the homologous series is ascended. In contrast to GuCl, guanadinium sulphate increased the affinity of 1-pentanol. Viewing alkanol affinity for the gel as an association process, these effects of guanidinium solutes are consistent with their effects on micelle formation by a non-ionic surfactant; GuCl destabilizes micellization whereas Gu_2SO_4 promotes it. Simple electrolytes also have similar effects on alkanol affinity; thus ammonium sulphate had an enhancing effect whereas the chaotropic anions I^- and SCN^- reduced affinity. The order of the anionic effects was that of the Hofmeister series. These results are consistent with the hypothesis that with water as solvent a hydrophobic interaction is the major determinant of the affinity of this gel for non-polar or weakly polar species such as the higher 1-alkanols.

INTRODUCTION

There is considerable evidence¹⁻⁴ that a hydrophobic interaction^{5,6} is involved in the affinity of the more highly cross-linked, but otherwise unsubstituted dextran gels for non-polar and weakly polar compounds when water is the solvent. The presence of water is probably mandatory since its replacement by formamide abolishes the affinity⁷. A brief report of the influence of water structure perturbants such as guanidinium salts and urea is also consistent with a hydrophobic interaction⁸. Further, a correlation between the effects of water structure perturbants on the affinity of the gel, on the one hand, and on micelle formation by a non-ionic surfactant, on the other, has been suggested⁹. This paper extends these observations and reports on

the influence on the distribution coefficient of higher alcohols in Sephadex G-10 when formamide is substituted for water and in aqueous systems containing both neutral and ionic perturbants. Finally, the effects of perturbants on the distribution coefficients of 1-pentanol and on micelle formation are compared.

EXPERIMENTAL

Materials

Sephadex® G-10 (Batch No. 2154) and dextran 500® were kindly donated by the manufacturer (Pharmacia Fine Chemicals, Uppsala, Sweden). The following ¹⁴C-labelled compounds were used: methanol (Radiochemical Centre, Amersham, U.K.), ethanol, 1-propanol, 1-butanol, 1-pentanol, acetone and ethylene glycol (NEN, Dreieich, F.R.G.) and 1-hexanol (Nuclear Equipment, Farmingdale, NY, U.S.A.). Tritiated water was also from the Radiochemical Centre. Guanidinium chloride was a specially purified grade for biochemistry (BDH, Poole, U.K.). Guanidinium sulphate (Fluka, Buchs, Switzerland) was crystallized twice from a methanol-benzene mixture. Formamide (Baker, Deventer, The Netherlands) was redistilled under low pressure before use. Anthrone (Merck-Schuchardt, München, F.R.G.) was crystallized twice from benzene. Cutscum® was from the Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other chemicals were of analytical grade.

Methods

Chromatography. The column dimensions were 60 × 1.0 cm I.D. and all aqueous solutions were made up in deionized water (Q-System, Millipore, Bedford, MA, U.S.A.). The volume of the loading solution which contained the test solute, dextran 500 and tritiated water was 0.5 ml. The linear flow-rate through the column never exceeded 3 cm/h.

Analysis. Dextran 500 and glucose were determined by an anthrone method¹⁰. ¹⁴C-labelled solutes and tritium were determined by liquid scintillation (Beckman CPM-200; Beckman, Fullerton, CA, U.S.A.).

Measurement of critical micelle concentration (cmc). Cutscum, a non-ionic surfactant (octylphenoxy polyethoxyethanol) with an undisclosed ethylene oxide (EO) number, was found to have a critical micelle concentration (cmc) in water of 0.224 ± 0.013 mg/ml, a value close to that of Triton X-100 (cmc = 0.208 ± 0.014 mg/ml) which has an EO number of 9–10.

The cmc was measured by a method¹¹ which utilizes the UV bathochromic shift (280–284.5 nm) undergone by the phenolic chromophore on transfer from an aqueous to the non-polar environment inside a micelle. The difference spectra were measured¹² in a spectrophotometer (PMQ II; Zeiss, F.R.G.). The critical micelle concentration was calculated, assuming the validity of the Bouguer-Beer law, as the intercept of the linear plots above and below the inflexion. The lines were calculated with a computer using an iterative procedure¹².

Calculations

The dynamic distribution coefficient, K_d , was calculated from the peak elution volumes of the test solute, V_e , of the void volume indicator dextran 500, V_0 , and of tritium of tritiated water, V_T , viz.

$$K_d = K_d^T \cdot \frac{V_e - V_0}{V_T - V_0} \quad (1)$$

where K_d^T , the distribution coefficient of tritium, is required to correct for the exchange between tritium and the hydroxyl hydrogens of the gel matrix; it can be determined from the difference between the elution volumes of the tritium and water labelled with non-exchanging isotopic oxygen (^{18}O)¹³. This batch of Sephadex G-10 had a K_d^T value of 1.091 in water.

In the experiments with formamide, tritium (tritiated water) was also used as an indicator, but in calculating the distribution coefficient no correction was made, $K_d^T(\text{F})$ being assumed to be unity which is certainly incorrect. This will give an underestimate of $K_d(\text{F})$ but values of ΔH° will not be affected. In Figs. 1, 2 and 4 the ordinate is therefore chosen as $\ln K$ to circumvent this problem; for aqueous solutions $\ln K \equiv \ln K_d$ whereas in the case of formamide $\ln K$ is the uncorrected value. Assuming that weights of water and formamide imbibed by the gel are in the same ratio as their dielectric constants (78/109), solute K_d values in formamide should be about 15% higher than those given in the text. This discrepancy does not however affect the validity of any of the conclusions drawn although there are small errors in the values of both ΔG° and ΔS° .

The standard transfer thermodynamic functions were calculated from the usual equations, *viz.*

$$\Delta G^\circ = -RT \ln K_d \quad (2)$$

$$\frac{d \ln K_d}{dT} = \frac{\Delta H^\circ}{RT^2} \quad (3)$$

and

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (4)$$

RESULTS

Fig. 1 shows that the increase in K_d value with increasing hydrocarbon chain length of a 1-alkanol is reversed when formamide is substituted for water. With the former solvent the progressive decline in the $\ln K_d$ value on increasing molecular size is characteristic of a molecular-sieve, or steric-exclusion, dominated partitioning process¹⁴.

It should be noted that the alcohol line in formamide in Fig. 1 is almost certainly located a little higher than shown. The reason for, and consequences of, this were discussed under *Calculations*.

Fig. 2 illustrates the $\ln K_d$ -reducing effects of formamide and aqueous solutions of urea and guanidinium chloride (GuCl). Of the last two, GuCl is more active, as in protein denaturation¹⁵. Although, on a concentration basis, urea and GuCl appear to be more active than formamide, it is doubtful whether such a quantitative comparison is valid since there is only one point for formamide, *i.e.*, a water-free state and the relationship may not be linear over the whole concentration range.

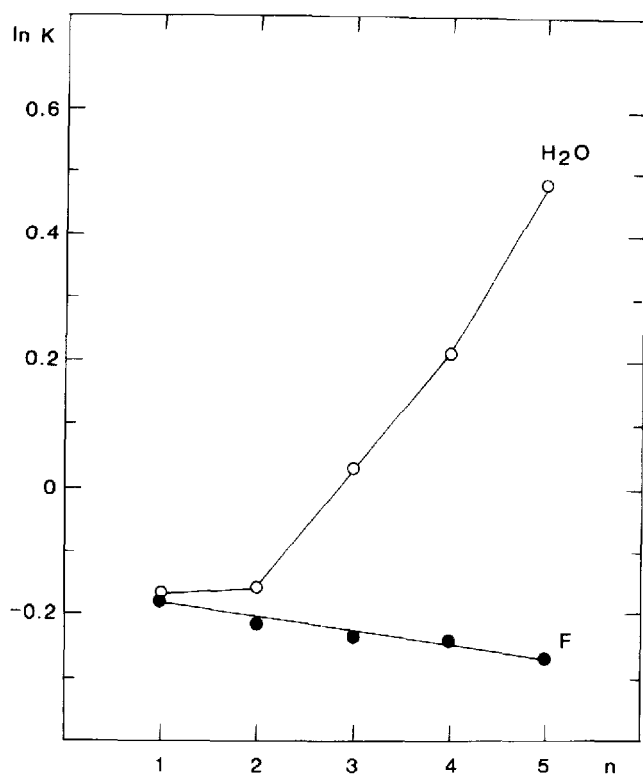


Fig. 1. $\ln K_d$ values of 1-alkanols with water or formamide (F) as eluent; n is the number of C atoms in the alcohol.

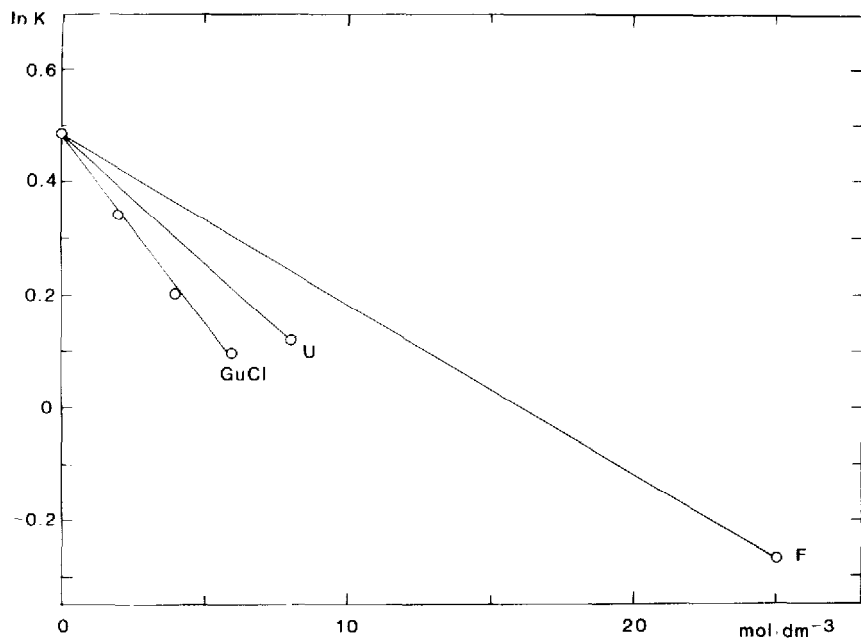


Fig. 2. $\ln K_d$ values of 1-pentanol in relation to the concentrations of aqueous solutions of urea (U) and guanidinium chloride (GuCl). The point F is for elution in pure formamide.

Fig. 3 shows the relationship between the $\ln K_d$ values of 1-alkanols, acetone, ethylene glycol and glucose and the GuCl concentration in the eluent when water is the solvent. As expected with the 1-alkanols, the K_d -reducing effect increases with the number of carbon atoms although there is no reduction in the case of the polar methanol. Fig. 3 also shows that the effect on acetone is roughly similar to that on 1-butanol. There was also little or no effect on ethylene glycol.

The temperature dependence of the $\ln K_d$ values of 1-pentanol in water, two aqueous GuCl concentrations and pure formamide is shown in Fig. 4. GuCl had a similar effect on 1-hexanol (data not shown). The values of the standard thermodynamic transfer functions at 25°C are also given in Table I. It should be borne in mind (Fig. 2) that the formamide concentration (25 mol/l) is much greater than those of GuCl and further that it is a water-free system.

Fig. 5 compares the effects on the K_d values of 1-pentanol of aqueous solutions of GuCl and guanidinium sulphate (Gu_2SO_4). The latter, in contrast to the chloride, increases the K_d value. This divergence of behaviour is also exhibited by different aqueous electrolytes (Fig. 6). The anion (*cf.*, GuCl and Gu_2SO_4) seems to exert the

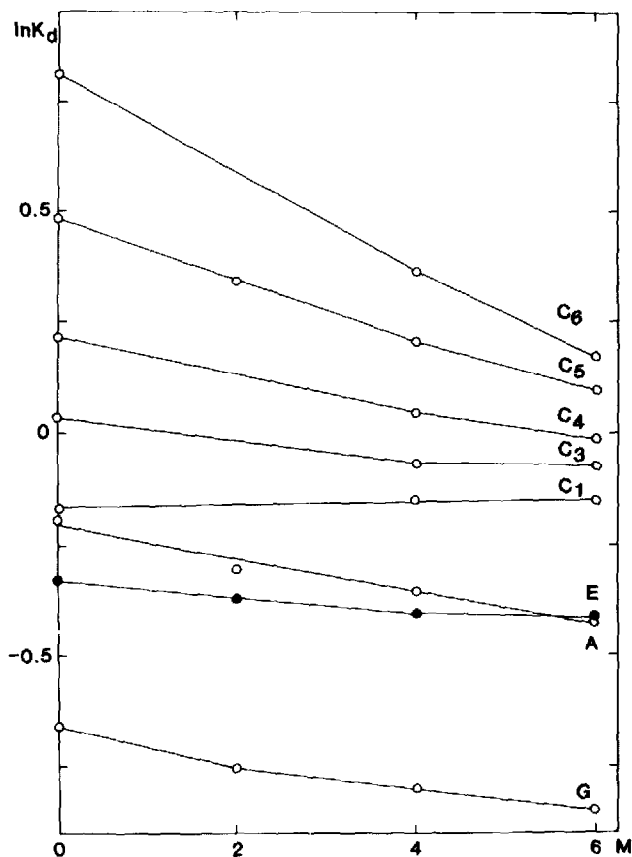


Fig. 3. $\ln K_d$ values of 1-alkanols and other solutes in relation to the concentration of guanidinium chloride. C₁-C₆ = Number of C atoms in 1-alkanol; E = ethylene glycol; A = acetone; G = glucose.

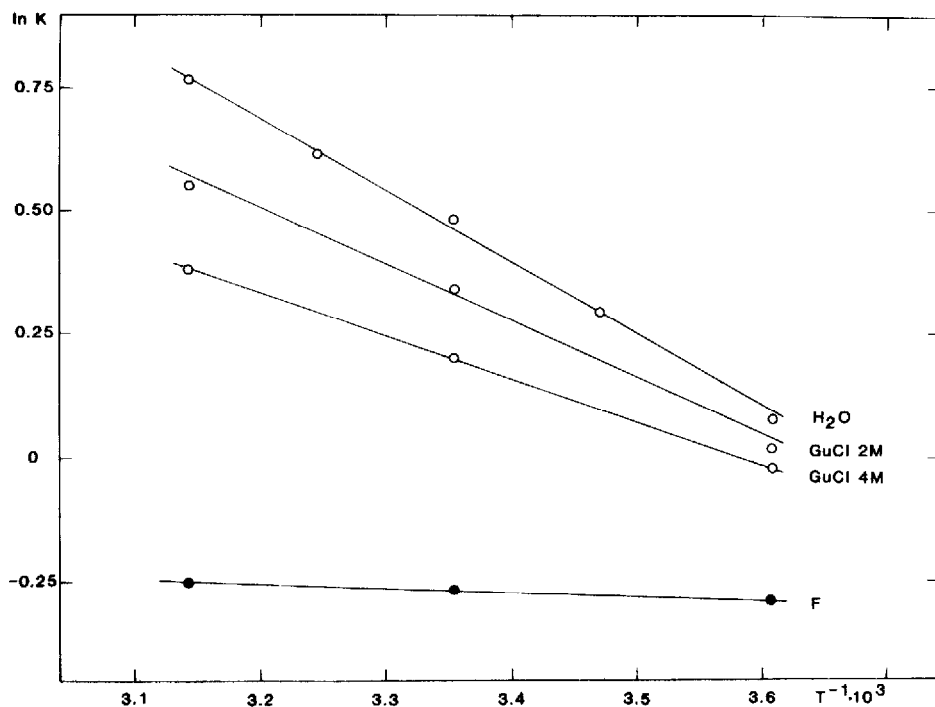


Fig. 4. Van 't Hoff plot of distribution coefficients of 1-pentanol in water, two aqueous guanidinium chloride solutions and formamide (F).

greater influence but the cations also contribute [*cf.*, for example, K_2SO_4 and $(NH_4)_2SO_4$].

Fig. 7 shows how some perturbants change the cmc of the non-ionic surfactant Cutscum.

Finally, Fig. 8 illustrates the relationship between the effects of simple electrolytes on the $\ln K_d$ values of 1-pentanol and their effects on the cloud point temperature of Triton X-100.

TABLE I

STANDARD THERMODYNAMIC TRANSFER FUNCTIONS AT 25°C

	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/mol · K)
<i>Pentanol</i>			
Water	-1.20	12.03	44.4
2 M GuCl	-0.85	9.48	34.6
4 M GuCl	-0.50	7.15	25.7
Formamide	+0.67	0.75	0.3
<i>Hexanol</i>			
Water	-2.00	14.48	55.3
6 M GuCl	-0.42	6.61	23.6

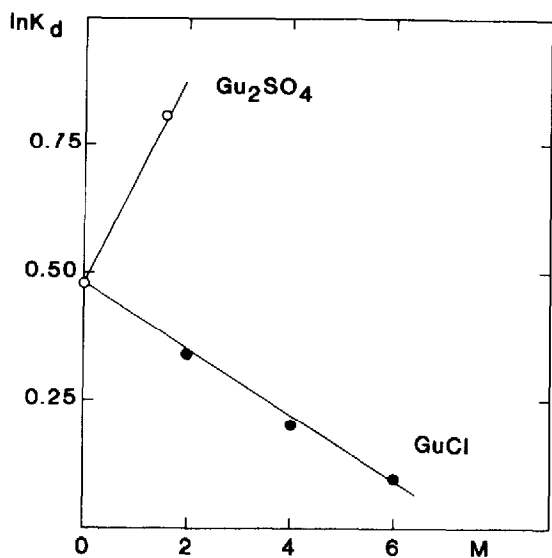


Fig. 5. $\ln K_d$ values of 1-pentanol in aqueous solutions of two guanidinium salts.

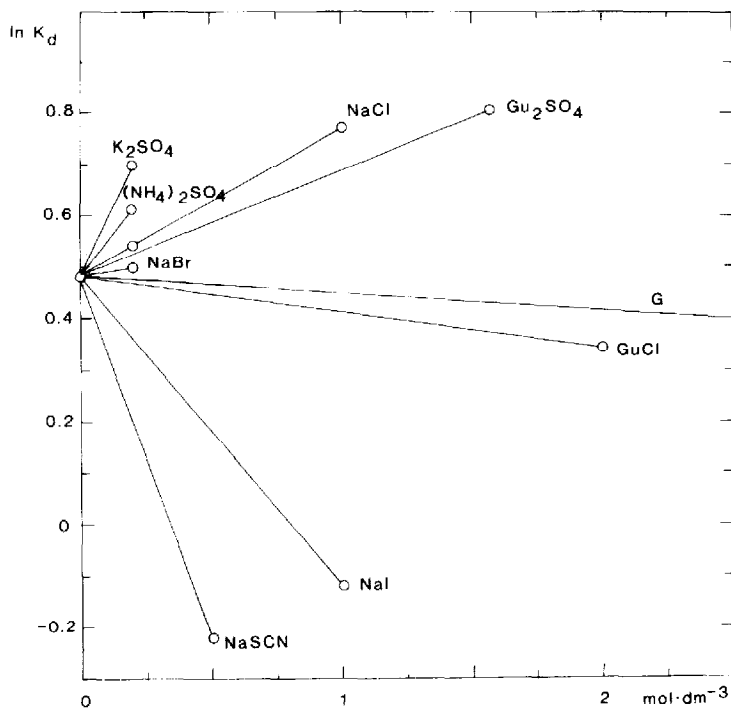


Fig. 6. $\ln K_d$ values of 1-pentanol when eluted with different electrolytes. The guanidinium points are from Fig. 5. The glucose line (G) is a linear interpolation from a measurement in 4 mol/l.

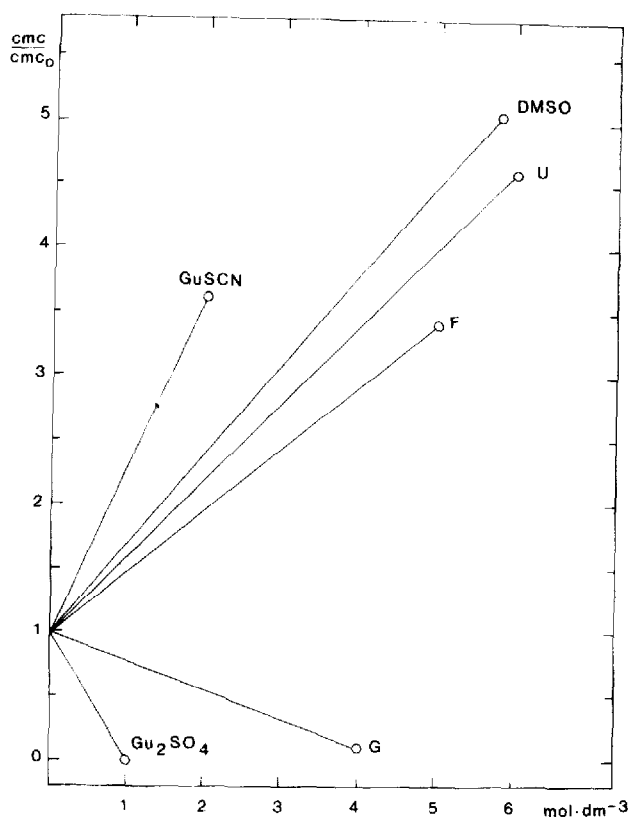


Fig. 7. Relative critical micelle concentrations (cmc/cmc_0) of Cutscum in aqueous solutions of dimethyl sulphoxide (DMSO), urea (U), formamide (F), glucose (G) and two guanidinium salts (GuX), cmc_0 is the cmc value in water.

DISCUSSION

The substitution of formamide for water (Fig. 1) does not affect all types of solute in the same way. In water, homologous series exhibit two limiting behavioural patterns². In a series such as the 1-alkanols, the higher members may be regarded as essentially non-polar due to the short range influence of the hydroxyl group⁵. Thus, the K_d difference between two consecutive members is due almost entirely to the addition of one non-polar methylene group. The fact alone that the gel has an affinity for the latter strongly indicates the probability of a hydrophobic interaction.

For a polar series such as the polyhydric alcohols, poly(ethylene oxide) or oligosaccharides the opposite is true; for these series partitioning is dominated by steric exclusion and the K_d value decreases with increasing molecular size².

The gel swells in formamide to roughly about the same, or a little greater, extent than in water. Although substitution of formamide for water should abolish hydrophobic interaction it would be expected to effect steric exclusion hardly at all. This is, in fact, what is observed. In Sephadex G-15 the slopes for the cellodextrins were thus of the same sign in both solvents, that in formamide being a little steeper,

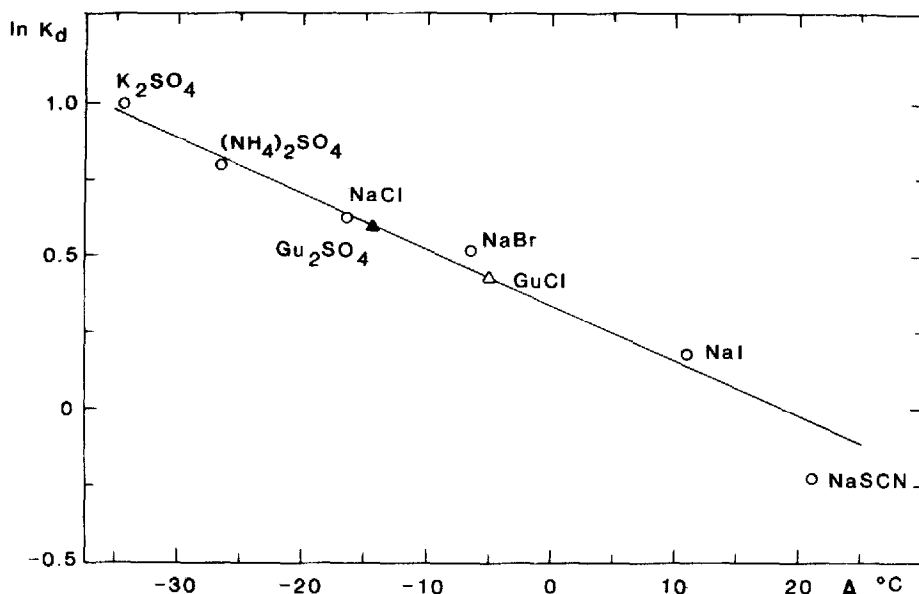


Fig. 8. The relationship between the effects of aqueous solutions of different electrolytes on the $\ln K_d$ values of 1-pentanol in Sephadex G-10 and on the cloud-point shift, $\Delta(^{\circ}\text{C})$, of Triton X-100. The latter values are from ref. 26. See text for further details.

i.e., in formamide the cellodextrin K_d values were a little lower than in water¹⁶. These small differences may be due to differences in the porosity of the gel in the two solvents and/or to small differences in the interaction energies, or differences in apparent solute size in the two solvents. Brown¹⁶ has pointed out that the intrinsic viscosity of glucose is quite different in water and formamide, being higher in the latter, thus indicating that the hydrodynamic volume of glucose is considerably larger in formamide than in water.

The fact that in formamide both ΔG° and ΔS° of 1-pentanol are greatly reduced is consistent with the suppression of a water-dependent hydrophobic interaction. As seen in Fig. 1, the difference in ΔG° between gels swollen in water and in formamide increases with the number of carbon atoms in the alcohol. Since the ΔG° values of the alcohols do not differ much in formamide, it seems reasonable to suppose that their ΔH° values will also be similar and thus, the higher the alcohol the greater will also be the change in ΔH° and ΔS° when water is replaced by formamide. Other non-aqueous polar solvents probably have similar effects on the partitioning of non-polar and weakly polar solutes. Thus, the polar aprotic solvent dimethylsulphoxide (DMSO) greatly reduced the affinity of some aromatic compounds¹⁷.

Fig. 3 indicates an analogous situation with aqueous GuCl solutions as regards ΔG° for the alcohols. The GuCl data, however, indicate that there is not a general relationship between the polarity of a solute and the change in ΔG° . Such a relationship holds for the alcohols, and acetone and ethylene glycol also behave as might be expected. The possible depressive effect of GuCl on glucose however appears to be anomalous and might reflect the presence of non-polar domains on the faces of the pyranose ring in its preferred chair (${}^4\text{C}_1$) conformation¹⁸.

Judging from the case of 1-pentanol, both concentrations of GuCl have a similar but less marked effect than pure formamide on both ΔG° and ΔH° ; the slopes of the lines in Fig. 4 are, in fact, $-\Delta H^\circ/R$.

The fact that both substitution of formamide for water and the addition of urea and GuCl have similar effects reinforces the argument that a hydrophobic interaction is involved in the affinity of the gels for non-polar solutes when water is the solvent. However, the actions of GuCl and urea are complex and are known to involve both hydrophobic and polar interactions and on that account considerable caution is warranted in interpreting their effects¹⁹⁻²².

However, as regards guanidinium salts there is a parallelism which must be regarded as strong support for a major contribution of a hydrophobic interaction to the gel affinity. There is thus a striking divergence between the effects of Gu_2SO_4 and GuCl, not only on the K_d value of 1-pentanol but also on micelle formation by the non-ionic surfactant Cutscum. This indicates the dominant role of SO_4^{2-} , and is consistent with the reported great effectiveness of this anion in salting out non-polar amino acid residues^{23,24}, in stabilizing the native form of penicillinase²⁵ and in reducing the cloud point temperature of the surfactant Triton X-100²⁶. Thus Gu_2SO_4 promotes both micellization (reduces cmc) and the affinity of the gel for 1-pentanol, whereas GuCl (and GuSCN) has the opposite effects.

Micelle formation like the affinity of the gel is an association process and like hydrophobic interaction both these processes appear to be entropy directed²³.

The effect of GuCl on the cmc is not shown in Fig. 7 but it is similar to, although somewhat less than that of GuSCN¹¹. Both formamide and urea also conform to the same pattern in that, like GuCl, they suppress micelle formation. Glucose is apparently an exception in that it reduces both cmc (Fig. 7) and (slightly) the K_d of 1-pentanol (Fig. 6). This anomaly may be, however, apparent rather than real in that the opposite effects of Gu_2SO_4 and GuCl may reflect similar orders of effectiveness rather than that a perturbant which raises cmc, reduces K_d and *vice versa*²⁷. Thus the order of effectiveness in raising the cmc is, for example, $\text{Gu}_2\text{SO}_4 < \text{glucose} < \text{GuCl}$ which is the same as their order of effectiveness in reducing K_d .

Apart from the influence of the guanidinium salts, it is almost certainly relevant that there is also a close parallelism between the effects of simple electrolytes on the affinity of the gel for a solute such as 1-pentanol and their effects on transitions of other macromolecular systems such as protein denaturation²⁴ and salting-in and salting-out phenomena²⁸⁻³⁰. Thus, at one end of the scale as mentioned above, SO_4^{2-} promotes the affinity of the gel for non-polar solutes and also stabilizes protein conformations, *i.e.*, has a salting-out effect. At the other end of the scale the large polarisable, so-called chaotropic anions such as SCN^- and I^- , which reduce the affinity of the gel for non-polar solutes, promote the hydration of non-polar groups, *i.e.*, they have a salting-in effect resulting in their increased aqueous solubility²⁵. The order of effect on the gel is thus the same as in several macromolecular systems, *i.e.*, the Hofmeister series³¹.

A recent quantitative study of the effect of simple electrolytes on the cloud point or upper consolute temperature of Triton X-100 underlines this problem²⁶. Shifts in cloud points, $\Delta(^{\circ}\text{C})$, were assigned to individual ions, NO_3^- arbitrarily being given the value zero, where $\Delta < 0$ indicates a lower cloud-point temperature, *i.e.*, salting-out effect. The calculated Δ values for the electrolytes included in this work

increase in the same order as the decreasing distribution coefficient of 1-pentanol in the gel, and further, the relative effectiveness of the electrolytes in these two systems appears to have a similar magnitude.

Fig. 8 shows the relationship between $\ln K_d$ of 1-pentanol (extra- or interpolated to the same electrolyte concentration, 0.5 mol/l, in Fig. 6) and the Δ values of the electrolytes from ref. 26. Although this relationship can only be regarded as tentative, it is possible to test its plausibility by estimating the Δ value of the guanidinium ion in GuCl and check the self-consistency of this value by use of Gu_2SO_4 . This was done in the following manner. The $\ln K_d$ of 1-pentanol was interpolated from Fig. 6 as 0.59 and 0.44 in 0.5 M Gu_2SO_4 and GuCl respectively. The latter value was used in Fig. 8 (open triangle) to estimate the Δ value of GuCl ($= -5$). Since the Δ value of Cl^- is -10.5 (ref. 26), the guanidinium ion has a value of $+5.5$. The Δ value of SO_4^{2-} is -25.5 , and, using the value of $+5.5$ for guanidinium, Gu_2SO_4 is therefore assigned a value of $-25.5 + 2(5.5) = -14.5$, a figure which is consistent with the effect on 1-pentanol affinity (filled triangle in Fig. 8).

NOTE ADDED IN PROOF

We should also like to thank the Editor for pointing out to us that, in addition to their effect in reducing the K_d values of weakly polar species such as the higher 1-alkanols, anions such as SCN^- or I^- have an affinity for the gel. This latter property is also shared by other poorly hydrated anions, e.g. ReO_4^- , ClO_4^- and AuCl_4^- .

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