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## INFLUENCE OF ADDITIVES TO THE ELUENT ON HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF SIMPLE COMPOUNDS

### III. THERMODYNAMICS OF INTERACTION OF *n*-ALCOHOLS WITH OCTYL-AGAROSE IN MIXTURES OF WATER WITH METHANOL, UREA OR ETHYLENE GLYCOL

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#### SUMMARY

Standard thermodynamic quantities for the interaction of *n*-alcohols with octyl-agarose in aqueous mixtures of methanol, ethylene glycol and urea are calculated from retention data at different temperatures. The use of Van 't Hoff plots is discussed. Results are compared with literature data and are interpreted with a simple model of hydrophobic hydration.

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#### INTRODUCTION

This paper is the third in a series on the influence of organic additives in hydrophobic interaction chromatography with octyl-agarose. So far, we have studied the properties of the layer of octylglycidyl groups (OG) at 25°C. It was concluded that the retention can be described by a liquid-liquid partition model. The layer of OG has liquid properties closely resembling those of *n*-octanol. The variation of the partition constant with the composition of the eluent is caused solely by the variation of the activity coefficient of the solute in the eluent<sup>1</sup>, even if the co-solvent is strongly sorbed by the stationary phase<sup>2</sup>.

The present paper describes the influence of the temperature on the chromatography of *n*-alcohols in aqueous mixtures of methanol, urea, ethylene glycol and, for the sake of comparison with (scarce) literature values<sup>3</sup>, in a single water-dimethylformamide (DMF) mixture. The first three are of interest in chromatography, as they have been used to elute sorbed protein<sup>4–6</sup>, and DMF has been proposed as a reference co-solvent in calorimetric determinations of enthalpies of solution<sup>7</sup>. Thermodynamic standard quantities can be calculated from the temperature dependence of the measured elution volumes. The standard states of the solutes in the layer of OG are not subject to variations of sorbed amounts of additive with temperature, or with eluent composition, because of the low affinity of these co-solvents for the layer of OG. The

decrease of the retention with increasing amounts of co-solvent can then be interpreted by a simple model, and the results can be compared with literature data on the vaporization of the *n*-alcohols from the partly aqueous solvents.

The thermodynamics of the interaction in an aqueous 25 mM phosphate buffer<sup>8</sup> has been described before. It was demonstrated that the values of  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Delta C_p^\circ$  show all the characteristics of the transfer of an apolar solute from water to an organic medium, closely resembling *n*-octanol.

## THEORETICAL

It is our aim to calculate values of  $\Delta G^\circ$ ,  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Delta C_p^\circ$  (henceforth denoted as  $\Delta X^\circ$ ) from the variation of the retention volumes with temperature and to interpret these data as a function of the amount of co-solvent in the eluent. First, a discussion about the difficulties and the reliability of the method is given.

It is obvious that the standard state of a solute in the stationary phase must preferably not depend on the possible variation with temperature of the sorbed amounts of co-solvent,  $W_{s,A}^\circ$  (grams of A per gram of OG groups). Such a variation would have a temperature-dependent impact on the retention volumes that would lead to corresponding trends in values of  $\Delta G^\circ/T$  as a function of  $1/T$  and, hence, to values of  $\Delta X^\circ$  that are difficult to interpret. It should be noted that such difficulties occur particularly with batch partition experiments in, *e.g.*, the octanol-water system. For in those systems, contrary to the chromatographic system with its bonded phase, the organic phase also may dissolve in the aqueous phase. Further, the experimental problems associated with batch partition experiments are much larger.

Fortunately, there exists a class of co-solvents that do not sorb on the layer of OG, *i.e.* electrolytes and highly polar organic substances such as sucrose and urea. In that case, the properties of the adsorbent resemble those of pure *n*-octanol at the same temperature, irrespective of the amount of co-solvent in the eluents, thus the same standard state in the stationary phase holds at all eluent compositions and temperatures. With methanol or ethylene glycol, however, where  $W_{s,A}^\circ$  is not exactly zero, the possible influence of a small amount of these co-solvents in the layer of OG needs careful attention before the results are interpreted (see Results and discussion).

The density,  $d_{OG}$ , of the stationary phase is another topic that deserves attention. In previous papers<sup>1,2</sup>, the mole fraction partition constant  $K_{X,B}$  has been proved suitable for the description of the chromatographic system at a constant temperature. The value of  $d_{OG}$  is eliminated in that quantity. However, the temperature dependence of  $-RT \ln K_{X,B}$  cannot be used for the calculation of readily interpretable values of  $\Delta X^\circ$ : the variations of the densities of the eluents with temperature and composition differ considerably from one eluent to another. This leads to artificial trends in values of  $\Delta X^\circ$ . Rather, it has been pointed out<sup>9</sup> that the molarity distribution constant,  $K_B$ , should be used. This implies that we have to face the problem of the unknown value of  $d_{OG}$ . Fortunately, it will be seen that various realistic assumptions about the value of  $d_{OG}$  lead to very small differences in the calculated values of  $\Delta X_{OG}^\circ$ . In the following paragraph, the standard free energy of transfer of 1 mole of B from an infinitely dilute solution in the eluent to the stationary phase,  $\Delta G_{OG}^\circ$ , is calculated, taking a small amount of sorbed co-solvent into account.

*Calculation of  $\Delta G_{OG}^{\circ}$* 

We assume that the chromatographic conditions do not differ significantly from the standard state of infinite dilution. The distribution constant  $K_B$  is given by

$$K_B = \frac{c_{B,og}}{c_{B,aq}} = \frac{n_{B,og}/V_{og}}{n_{B,aq}/V_{aq}} \quad (1)$$

Here,  $c_B$  denotes the concentration of a solute B ( $\text{mol l}^{-1}$ ),  $V_{og}$  and  $V_{aq}$  are the volumes of the stationary and the mobile phase in the column, respectively, and  $n_B$  denotes the number of moles of B.  $V_{og}$  can be approximated as the sum of the volumes of the pure constituents: octylglycidyl ( $V_{OG}$ ) and sorbed additive ( $V_{A,or}$ ). If we substitute in eqn. 1 the expression for the capacity ratio,  $k_B$

$$k_B = \frac{n_{B,og}}{n_{B,aq}} = \frac{V_e - V^{\circ}}{V^{\circ}} \quad (2)$$

we have

$$K_B = \frac{V_e - V^{\circ}}{V_{OG} + V_{A,or}} \quad (3)$$

Here,  $V^{\circ}$  denotes the elution volume of a supposedly unretarded compound (hence  $V^{\circ} = V_{aq}$ , see ref. 1) and  $V_e$  that of B. Substitution of the expression for the specific retention volume<sup>1</sup>,  $V_g = (V_e - V^{\circ})/w_{OG}$  ( $w_{OG}$  denotes the weight of OG groups in the column) and transformation of volumes into amounts yield an expression for  $K_B$ , from which  $\Delta G_{OG}^{\circ}$  can be calculated as

$$\Delta G_{OG}^{\circ} = -RT \ln V_g + RT \ln [d_{OG}^{-1} + W_{s,A} d_A^{-1}] \quad (4)$$

Here,  $d_{OG}$  denotes the density of the layer of OG and  $d_A$  that of the pure additive ( $\text{g ml}^{-1}$ ). Thus  $\Delta G_{OG}^{\circ}$  can be calculated from specific retention volumes at different temperatures, provided that values of  $W_{s,A}$ ,  $d_A$  and  $d_{OG}$  are known at those temperatures. It follows from eqn. 4 that the methylene increments of  $\Delta G_{OG}^{\circ}$  can be obtained from specific retention volumes only:

$$\Delta G_{OG,CH_2}^{\circ} = -RT \ln V_{g,n}/V_{g,n-1} \quad (5)$$

where  $n$  denotes the number of carbon atoms. (Of course, this elimination of  $d_{OG}$  and  $W_{s,A}$  does not lead to the nullification of the influence of sorbed additive on the methylene increments.

A particularly simple situation exists if the sorbed amounts are too small to have any influence on the values of  $\Delta G_{OG}^{\circ}$ . In that case, ambiguity about  $d_{OG}$  can be avoided by calculating the change of  $\Delta G_{OG}^{\circ}$  with changing eluent composition, from retention volumes only. From this change, and literature data on the free energy of hydration, the free energy of solvation in the partly aqueous eluent can then be calculated. The calculations proceed as follows. It holds that

$$\Delta G_{OG}^{\circ} = \Delta G_s^{\circ} - \Delta G_h^{\circ} \quad (6)$$

Here,  $\Delta G_s^0$  denotes the free energy of solvation of 1 mole of solute in the layer of OG (transfer vacuum  $\rightarrow$  layer of OG) and  $\Delta G_h^0$  that in the partly aqueous eluent. Note that  $\Delta G_s^0$  in eqn. 6 is independent of the eluent composition in the considered case. From eqn. 6

$$\Delta G_h^0 = \Delta G_h^{*0} - \Delta G_{OG}^{*0} + \Delta G_{OG}^{*0} \quad (7)$$

where the asterisks refer to the values in a pure aqueous eluent, and substitution of eqn. 4 transforms this into

$$\Delta G_h^0 = \Delta G_h^{*0} + RT \ln (V_g/V_g^*) \quad (8)$$

## EXPERIMENTAL

The experimental procedure has been described before<sup>1,8</sup>. Special care was given to preventing vaporization of the eluent constituents.

The elution peaks were symmetrical at temperatures above 0°C. Below this temperature, in aqueous methanol,  $C_A = 0.098$  (grams of A per gram of eluent) peak shape changed drastically and serious tailing was observed. Here, the layer of OG might lose its liquid properties. Such phase transitions in bonded layers have been observed before<sup>10,11</sup> in reversed-phase high-performance liquid chromatography.

With two eluents, *i.e.* the highest concentration of urea and ethylene glycol at  $t > 25^\circ\text{C}$ , the baseline stability was problematic. We were forced to increase the sample dose to 1 mg. This hardly affected the accuracy. However, measurements with higher homologues than *n*-octanol were not possible owing to long-term drifts.

## RESULTS AND DISCUSSION

Table I lists values of  $V_g$  (millilitres per gram of OG groups) of *n*-alcohols on octyl-Sepharose CL-4B at various temperatures. The precision of these values is estimated to be *ca.* 0.4 ml g<sup>-1</sup> or 0.8%, for small and large values of  $V_g$ , respectively. Control experiments with unsubstituted Sepharose showed no significant contribution of agarose to the retention. Before applying eqns. 4–8, we shall first discuss the possible influence of small amounts of additive in the layer of OG.

### *Influence of sorption*

As we stated before, with some organic co-solvents  $W_{s,A}^0$  is not exactly zero. Of the four additives, methanol probably has the largest affinity for the layer of OG. This can be inferred from the values of the octanol–water distribution constants: methanol, 0.15<sup>12</sup>; DMF, 0.10<sup>13</sup>; urea, 0.08<sup>14</sup>; and ethylene glycol, 0.012<sup>15</sup>. Therefore, the following discussion about methanol *a fortiori* holds for the other co-solvents.

The influence of sorption may become manifest in two different ways: (i) in the change of the amount of stationary phase with temperature (this effect is accounted for in eqn. 4); (ii) in the change of the nature of the layer of OG.

(i) From the data in Table I, for the model substance *n*-octanol in methanol–water eluents, we used eqn. 4 to calculate different estimates of  $\Delta G_{OG}^0/T$  at each

TABLE I  
SPECIFIC RETENTION VOLUMES OF *n*-ALCOHOLS ON OCTYL-SEPHAROSE CL-4B

Additive ( <i>A</i> )	$C_A$	<i>t</i> (°C)	Number of carbon atoms in model substance					
			5	6	7	8	9	10
Methanol	0.000	4.2	12.8	46.3	172	641		
		10.4	13.9	50.9	189	700		
		18.0	15.9	57.9	211	775		
		25.7	17.5	62.6	225	814		
		32.9	18.4	66.1	233	827		
		40.3	18.9	68.5	239	832		
Methanol	0.098	5.6	11.7	40.7	148	532	1869	
		9.8	13.6	43.3	152	543	1897	
		18.2	13.1	44.6	156	548	1897	
		26.7	13.4	46.4	158	534	1793	
		34.4	14.3	47.2	157	514	1683	
		42.8	13.3	46.1	148	480	1544	
Methanol	0.199	3.4	9.2	36.9	126	426	1407	
		10.4	10.7	38.3	120	397	1255	
		18.0	12.8	37.7	117	367	1124	
		25.7	11.8	36.8	112	339	996	
		33.1	11.0	33.7	103	303	879	
		40.4	11.6	32.8	95	272	783	
Methanol	0.303	4.9	9.7	26.9	75.9	219	614	1697
		9.2	9.8	26.2	73.3	205	567	1560
		18.0	10.5	23.9	66.9	179	470	1247
		26.7	6.8	21.7	58.3	148	374	940
		34.6	8.3	20.7	51.4	126	314	738
		43.2	7.3	19.7	46.2	107	252	605
Methanol	0.414	1.9		15.1	35.1	84.2	198	457
		9.2		13.7	32.3	73.8	170	378
		18.0		14.4	29.9	67.5	146	312
		25.4		12.1	26.4	57.5	123	259
		33.9		13.2	22.8	48.1	102	213
		41.7		10.6	20.8	42.7	84	171
Urea	0.059	5.5	13.3	49.2	174	674		
		11.5	14.6	52.0	187	729		
		16.3	15.9	55.1	204	753		
		22.6	17.3	58.3	210	784		
		29.8	17.9	62.4	219	798		
		39.4	19.7	66.0	221	798		
Urea	0.172	3.6	12.0	40.9	157	583		
		12.4	13.1	45.3	168	603		
		18.8	14.0	49.5	178	613		
		26.3	16.5	51.5	182	634		
		33.4	15.5	53.4	183	632		
		44.3	14.9	51.7	177	610		

(Continued on p. 24)

TABLE I (continued)

SPECIFIC RETENTION VOLUMES OF *n*-ALCOHOLS ON OCTYL-SEPHAROSE CL-4B

Additive (A)	$C_A$	$t$ (°C)	Number of carbon atoms in model substance					
			5	6	7	8	9	10
Urea	0.279	3.2	12.0	37.3	145	515		
		10.4	12.3	42.0	154	524		
		18.0	13.5	44.5	156	526		
		25.0	13.8	45.2	156	535		
		33.3	13.7	46.3	155	511		
		40.7	13.7	45.9	154	503		
Urea	0.380	4.9	—	34.1	118	387		
		12.1	12.3	36.8	123	392		
		18.4	13.8	38.7	127	399		
		25.2	13.4	40.8	130	387		
		33.7	14.7	44.4	128	—		
		41.1	—	40.9	126	369		
Ethylene glycol	0.153	3.6	11.1	47.3	175	641		
		11.3	11.9	49.9	185	674		
		18.1	13.6	52.6	195	679		
		25.3	15.3	55.7	192	658		
		34.1	15.5	56.2	190	645		
		42.6	17.5	55.8	187	619		
Ethylene glycol	0.299	0.0	12.9	47.5	173	621		
		6.3	13.2	47.9	167	583		
		13.5	13.5	47.3	161	558		
		19.6	13.9	47.1	158	514		
		26.8	14.1	46.2	150	475		
		33.9	13.2	44.2	139	—		
40.6	13.2	41.3	127	390				
Ethylene glycol	0.440	0.8	13.7	44.2	140	464		
		6.8	12.8	41.8	131	416		
		12.7	12.6	40.0	122	382		
		20.7	11.6	37.4	111	336		
		29.8	11.4	35.1	100	282		
		39.4	11.6	31.5	84.0	230		
DMF	0.331	4.0		18.9	62.0	175		
		11.2		17.5	55.2	160		
		19.8		17.5	51.8	139		
		26.2		16.9	46.3	122		
		33.4		16.0	41.5	109		
		41.2		13.6	35.9	92.8		

experimental temperature by making various estimates of the values of  $d_{OG}$ ,  $d_A$  and  $W_{s,A}^0$  as a function of  $T$ . From the estimates of  $\Delta G_{OG}^0/T$  we calculated  $\Delta H_{OG}^0$ ,  $T\Delta S_{OG}^0$  and  $\Delta C_{p,OG}^0$  as before<sup>8</sup>. The results, presented in Fig. 1, show the small influence of  $d_{OG}$ ,  $d_A$  and  $W_{s,A}^0$ . Clearly, the variation of  $-R \ln V_g$  with  $T$  and with

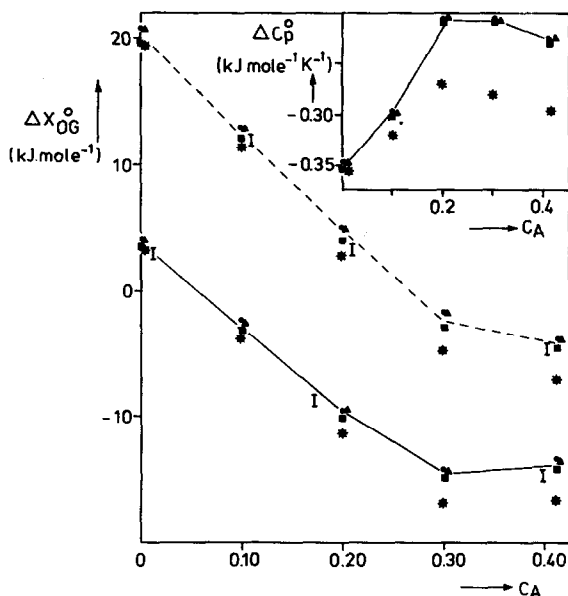


Fig. 1. Influence of model parameters on calculated thermodynamic standard quantities of *n*-octanol in methanol-water mixtures.

(a)	$d_{OG}$	$\frac{\partial d_{OG}}{\partial T}$	$W_{s,A}^{\circ}$	$\frac{\partial W_{s,A}^{\circ}}{\partial T}$	$d_A$	$\frac{\partial d_A}{\partial T}$
■	0.89 <sup>(b)</sup>	(c)	Refs. 1 and 2 (20°C)	0 <sup>(d)</sup>	0.8	Ref. 19
▲	1	0	Refs. 1 and 2 (20°C)	0	1	0
●	1	0	0	0	1	0
*	0.89	(c)	Refs. 1 and 2	(e)	0.8	Ref. 19

(a) Full lines =  $\Delta H_{OG}^{\circ}$ ; broken lines =  $T\Delta S_{OG}^{\circ}$ .

(b) Density of octylglycidyl ether at 25°C<sup>16</sup>.

(c) Values for octanol, ref. 19.

(d) The value of  $W_{s,A}^{\circ}$  is probably independent of  $T$ . This follows from the constant value of the activity coefficient of methanol in all eluents between 40°C and 100°C (calculated from  $P$ - $X$  diagrams<sup>17,18</sup>) and from the value of the activity coefficient of methanol in the layer of OG (i.e., near unity<sup>1</sup>). Both are not expected to change much from 40°C to 5°C, and hence  $W_{s,A}^{\circ}$  can be expected to be constant.

(e) An unrealistic, very large fluctuation of  $W_{s,A}^{\circ}$  with  $T$  is assumed:  $W_{s,A}^{\circ}(T^{\circ}\text{C}) = W_{s,A}^{\circ}(20^{\circ}\text{C}) \times 2^{\frac{T-20}{20}}$ , i.e. a doubling of  $W_{s,A}^{\circ}$  per 20°C temperature increase.

$C_A$  outweighs that of  $d_{OG}$ ,  $d_A$  and  $W_{s,A}^{\circ}$ . Only an unrealistic change of  $W_{s,A}^{\circ}$  with  $T$  leads to different values, but even they show the same trend with eluent composition. These results lead us to neglect the sorbed amounts of co-solvent and to take  $d_{OG} = 1$ , which implies dropping the term  $RT \ln [d_{OG}^{-1} + W_{s,A}^{\circ} d_A^{-1}]$  from eqn. 4.

(ii) The possibility that changing amounts of sorbed co-solvent as a function of the eluent composition, and especially of the temperature, contribute to the change of retention volumes must be considered in spite of the small values of  $W_{s,A}^{\circ}$ . Whether

this occurs can be investigated with eqn. 6, provided that values of  $\Delta G_{OG}^{\circ}$  and  $\Delta G_h^{\circ}$  are known, as a function of the eluent composition. In that case,  $\Delta G_s^{\circ}$  can be calculated and tested for being constant. Values of  $\Delta G_{OG}^{\circ}$  have been dealt with in (i). They are known but for a small constant term arising from  $d_{OG}$ . Values of  $\Delta G_h^{\circ}$  can be calculated as follows. Assuming that the vapour of the solute behaves ideally and that its activity coefficient in the eluent equals that of a saturated solution in the eluent<sup>1</sup>,  $\Delta G_h^{\circ}$  follows from the ratio of the number density of the solute in the vapour phase and in the saturated eluent. It holds than that

$$\Delta G_s^{\circ} = \Delta G_{OG}^{\circ} - RT \ln \frac{RTS}{10^{-3} a P^{\circ} M_B} \quad (9)$$

where  $M_B$  denotes the molecular weight of the solute,  $a$  is its activity when saturated with the eluent and  $S$  is the solubility ( $\text{g l}^{-1}$ ) of the solute in the eluent, and  $P^{\circ}$  (Pa) is the vapour pressure of the pure solute.

We performed this calculation for *n*-octanol in methanol–water eluents at 25°C using the data from a previous paper<sup>1</sup>. Results, listed in Table II, show that  $\Delta G_s^{\circ}$  is constant within *ca.* 0.3 kJ mol<sup>-1</sup>.

TABLE II

FREE ENERGIES OF TRANSFER AND OF SOLVATION FOR *n*-OCTANOL IN METHANOL–WATER MIXTURES

$C_A$ ( $\text{g g}^{-1}$ )	$\Delta G_{OG}^{\circ*}$ ( $\text{kJ mol}^{-1}$ )	$S^{**}$ ( $\text{g l}^{-1}$ )	$a^{**}$	$\Delta G_s^{\circ***}$ ( $\text{kJ mol}^{-1}$ )
0.000	-16.60	0.572	0.79	-34.3
0.098	-15.59	0.730	0.72	-34.2
0.199	-14.44	1.16	0.64	-34.4
0.303	-12.48	2.57	0.57	-34.7
0.413	-9.61	6.52	0.48	-34.6

\* Eqn. 4 with  $V_g$  from Table I,  $d_{OG} = 1$  and  $W_{s,A}^{\circ} = 0$ .

\*\* See comments in ref. 1.

\*\*\* Eqn. 9,  $P^{\circ}$  from ref. 20.

With enthalpy values another test can be performed, also for *n*-octanol. The value of  $\Delta H_s^{\circ}$  ( $C_A = 0$ ), *i.e.* -70 kJ mol<sup>-1</sup>, was obtained with the enthalpy equivalent of eqn. 6 from the value of  $\Delta H_{OG}^{\circ}$  (Fig. 1) and the literature value of  $\Delta H_h^{\circ}$ , both in water<sup>20</sup>. The value of  $\Delta H^{\circ}$  for solvation of *n*-octanol in pure methanol is -69 kJ mol<sup>-1</sup> (found by extrapolation of literature values of lower alcohols with constant methylene increments<sup>21</sup>). As these values are nearly identical, the presence of a small amount of methanol in the layer of OG at higher values of  $C_A$  is not expected to influence  $\Delta H_s^{\circ}$  much.

We conclude that  $\Delta X_s^{\circ}$  does not depend on the eluent composition. This means that the variation of  $\Delta X_{OG}^{\circ}$ , and that of  $\Delta X_h^{\circ}$ , with eluent composition, are identical. Our chromatographic data on  $\Delta X_{OG}^{\circ}$  can thus be compared with literature data on  $\Delta X_h^{\circ}$  obtained by classical methods. The methods are complementary, as the chromatographic method works best with higher homologues that are well retained on



the octyl-agarose column, whereas classical methods work best with lower homologues that are well soluble in partly aqueous solvents and have a high volatility.

#### *Influence of the temperature on the retention volumes*

Fig. 2 presents a Van't Hoff plot ( $\Delta G_{OG}^{\circ}/T = -R \ln V_g$  vs.  $T^{-1}$ ) for *n*-octanol. The remarkable behaviour of pure water, especially at low temperatures, is clearly illustrated by this Figure. It is seen that the increase of the retention with temperature in pure water changes rapidly to the opposite behaviour if co-solvent (except urea) is added.

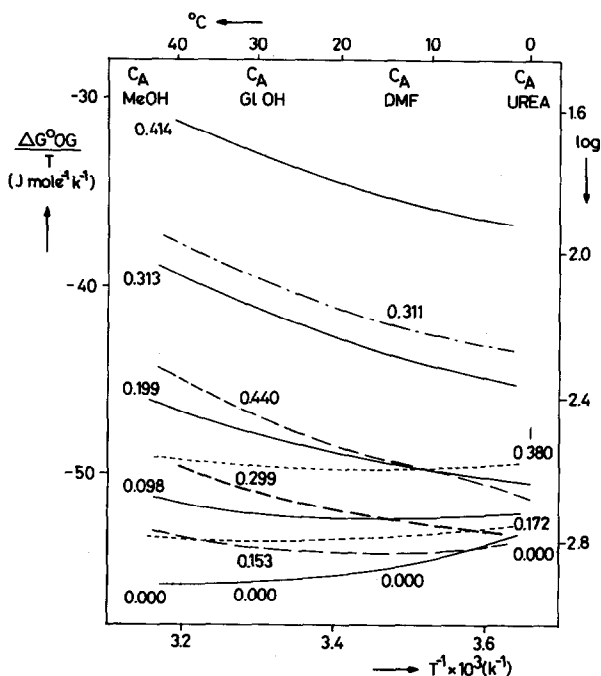


Fig. 2. Van't Hoff plot for *n*-octanol. Eluents: methanol (MeOH)-water = —; ethylene glycol (GlOH) = ---; urea = .....; DMF = -.-.-. Values of  $C_A$  (weight fraction of additive in the eluent) are indicated in the figure.

#### *Thermodynamics of the retention process*

Values of  $\Delta X_{OG}^{\circ}$  at 25°C were calculated as before<sup>8</sup>. They are listed in Table III. From the precision of the experimental data, an error of  $\pm 0.07$  kJ mol<sup>-1</sup> K<sup>-1</sup> can be predicted in  $\Delta G_{OG}^{\circ}/T$  for values of  $V_g > 100$  ml g<sup>-1</sup>. Table III shows that  $S_y$  values resulting from the statistical treatment are indeed of this order of magnitude.

The methylene increments of values of  $\Delta X_{OG}^{\circ}$  in Table III are independent of the chain length of the alcohols, within experimental error. More accurate values of  $\Delta X_{OG,CH_2}^{\circ}$  were established as follows. Values of  $\Delta G_{OG,CH_2}^{\circ}$  were calculated from the ratio of  $V_{g,n}$  and  $V_{g,n-2}$  with  $n = 8$  or 9, if measured (eqn. 5). Values of  $\Delta X_{OG,CH_2}^{\circ}$  were calculated from a Van't Hoff plot. They are listed in Table IV.

TABLE III

VALUES OF  $\Delta X_{OG}^{\circ}$  AT 25°C FOR INTERACTION OF *n*-ALCOHOLS WITH OCTYL-AGAROSE IN VARIOUS ELUENTS

<i>A</i> ( <i>C<sub>A</sub></i> )	<i>n</i>	$\Delta C_{p,OG}^{\circ}$	$\Delta H_{OG}^{\circ}$	$T\Delta S_{OG}^{\circ}$	$\Delta G_{OG}^{\circ}$	<i>s</i> *	<i>s<sub>y</sub></i> **
Methanol (0.000)	5	0***	8.2	15.2	-7.0	0.8	0.28
	6	-0.27	7.0	17.3	-10.24	0.2	0.06
	7	-0.30	5.5	19.0	-13.41	0.1	0.05
	8	-0.35	4.1	20.7	-16.60	0.2	0.06
Methanol (0.098)	5	0***	2.1	8.5	-6.4	1.3	0.49
	6	-0.24	2.0	11.5	-9.51	0.3	0.10
	7	-0.28	-0.3	12.3	-12.55	0.2	0.06
	8	-0.29	-2.5	13.1	-15.59	0.1	0.02
	9	-0.35	-4.5	14.1	-18.61	0.2	0.06
Methanol (0.199)	5	0***	3.1	9.1	-6.0	2.3	0.86
	6	-0.31	-4.0	5.0	-8.93	0.4	0.15
	7	-0.22	-6.1	5.6	-11.68	0.3	0.10
	8	-0.21	-9.5	5.0	-14.44	0.2	0.05
	9	-0.10	-11.8	5.4	-17.13	0.1	0.03
Methanol (0.303)	5	0***	-6.3	-1.1	-5.3	2.9	1.10
	6	0.07	-6.1	1.6	-7.70	0.2	0.09
	7	-0.18	-10.2	0.0	-10.19	0.3	0.12
	8	-0.21	-14.3	-1.8	-12.48	0.3	0.10
	9	-0.22	-17.5	-2.7	-14.82	0.2	0.08
	10	-0.19	-20.7	-3.6	-17.12	0.6	0.21
Methanol (0.414)	6	0***	-5.2	1.1	-6.29	1.7	0.65
	7	-0.22	-10.7	-2.6	-8.14	0.3	0.12
	8	-0.23	-13.4	-3.8	-9.61	0.5	0.14
	9	-0.28	-16.7	-4.8	-11.95	0.3	0.10
	10	-0.20	-18.5	-4.7	-13.81	0.3	0.12
	Urea (0.059)	5	-0.23	7.5	14.6	-7.10	0.4
6	-0.08	6.2	16.3	-10.13	0.2	0.05	
7	-0.35	4.1	17.4	-13.31	0.3	0.10	
8	-0.30	2.6	19.1	-16.55	0.1	0.03	
Urea (0.172)	5	0***	4.6	11.2	-6.62	1.8	0.65
	6	-0.34	3.8	13.6	-9.72	0.3	0.11
	7	-0.28	1.7	14.6	-12.89	0.2	0.07
	8	-0.17	0.7	16.7	-15.97	0.2	0.09
Urea (0.279)	5	0***	3.0	9.4	-6.45	1.5	0.54
	6	-0.34	2.4	11.9	-9.49	0.3	0.10
	7	-0.19	0.2	12.7	-12.53	0.3	0.09
	8	-0.16	-1.1	14.4	-15.53	0.2	0.09
Urea (0.380)	5	0***	5.2	11.7	-6.51	1.9	0.35
	6	-0.30	3.6	12.8	-9.26	0.8	0.27
	7	-0.24	0.7	12.8	-12.05	0.1	0.05
	8	-0.18	-1.5	13.3	-14.79	0.2	0.08

TABLE III (continued)

VALUES OF  $\Delta X_{\text{OG}}^{\circ}$  AT 25°C FOR INTERACTION OF *n*-ALCOHOLS WITH OCTYL-AGAROSE IN VARIOUS ELUENTS

<i>A</i> ( <i>C<sub>A</sub></i> )	<i>n</i>	$\Delta C_{p,\text{OG}}^{\circ}$	$\Delta H_{\text{OG}}^{\circ}$	$T\Delta S_{\text{OG}}^{\circ}$	$\Delta G_{\text{OG}}^{\circ}$	<i>s</i> *	<i>s<sub>y</sub></i> **
Ethylene glycol (0.153)	5	0***	8.5	15.2	-6.63	0.8	0.30
	6	-0.20	2.8	12.7	-9.92	0.3	0.10
	7	-0.26	0.4	13.4	-13.05	0.3	0.10
	8	-0.24	-1.6	14.6	-16.13	0.3	0.10
Ethylene glycol (0.299)	5	-0.23	-0.8	5.7	-6.50	0.4	0.15
	6	-0.22	-3.4	6.1	-9.50	0.2	0.07
	7	-0.23	-6.4	6.1	-12.43	0.2	0.10
	8	-0.21	-9.2	6.1	-15.32	0.2	0.07
Ethylene glycol (0.440)	5	-0.23	-2.0	4.1	-6.08	0.4	0.13
	6	-0.08	-6.5	2.4	-8.89	0.2	0.07
	7	-0.20	-10.2	1.3	-11.53	0.2	0.09
	8	-0.22	-14.0	0.3	-14.20	0.2	0.08
DMF	6	-0.35	-6.5	0.5	-7.01	0.8	0.28
	7	-0.24	-11.0	-1.5	-9.55	0.4	0.15
	8	-0.22	-13.1	-1.1	-12.00	0.2	0.08

\* *s* denotes the error in values of  $\Delta H_{\text{OG}}^{\circ}$  and  $T\Delta S_{\text{OG}}^{\circ}$  (kJ mol<sup>-1</sup>).\*\* *s<sub>y</sub>* denotes the error in a single value of  $\Delta G^{\circ}/T$ .\*\*\*  $\Delta C_p^{\circ}$  was not evaluated from the data, but arbitrarily put equal to zero.

TABLE IV

VALUES OF METHYLENE INCREMENTS OF  $\Delta X_{\text{OG}}^{\circ}$  AT 25°C

Eluent	<i>c<sub>A</sub></i>	$\Delta C_{p,\text{CH}_2}^{\circ}$	$\Delta H_{\text{CH}_2}^{\circ}$	$T\Delta S_{\text{CH}_2}^{\circ}$	$\Delta G_{\text{CH}_2}^{\circ}$	<i>s</i>	<i>s<sub>y</sub></i>
Methanol	0.000	-0.04	-1.48	1.70	-3.18	0.06	0.02
	0.098	-0.04	-2.09	0.94	-3.03	0.16	0.06
	0.199	+0.06	-2.83	0.10	-2.93	0.10	0.04
	0.303	-0.02	-3.70	-1.36	-2.34	0.13	0.05
	0.414	-0.01	-2.84	-0.92	-1.92	0.17	0.06
Urea	0.059	-0.12	-1.82	1.39	-3.20	0.14	0.04
	0.172	+0.09	-1.55	1.55	-3.10	0.14	0.05
	0.279	+0.09	-1.77	1.26	-3.03	0.21	0.08
	0.380	+0.06	-2.21	0.61	-2.82	0.20	0.06
Ethylene glycol	0.153	-0.02	-2.17	0.93	-3.10	0.25	0.09
	0.299	+0.01	-2.84	0.08	-2.92	0.12	0.05
	0.440	-0.03	-3.56	-0.92	-2.64	0.08	0.03
DMF	0.331	+0.05	-3.33	-0.82	-2.50	0.44	0.16

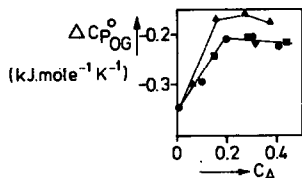


Fig. 3. Change of heat capacity values with eluent composition. Symbols: ● = methanol; ■ = ethylene glycol; ▲ = urea; ▼ = DMF.

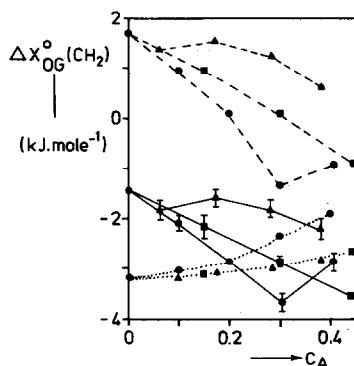


Fig. 4. Thermodynamics of the interaction of a methylene group with the octylglycidyl layer. Symbols as in Fig. 3, plus free energy = .....; enthalpy = ———; entropy = - - - -.

Fig. 3 shows values of  $\Delta C_{p,OG}^{\circ}$  for *n*-octanol as a function of  $C_A$ , and the influence of co-solvents on the methylene increments of free energy, enthalpy and entropy is given in Fig. 4.

A comparison with recalculated literature data is shown in Fig. 5. Here, we plotted our values of  $\Delta H_n^{\circ}$  (open symbols) together with recalculated data from Rouw and Somsen<sup>3</sup> on the heats of solution of some lower alcohols in mixtures of DMF and water. It can be seen that the agreement between the two sets of data is good.

A graphical representation of eqn. 6 for methanol–water eluents is given in Fig. 6, and Fig. 7 presents the results for eluents with ethylene glycol and urea. In the latter figure, values of  $\Delta H_n^{\circ}$  for *n*-butanol, recalculated from data of Nwankwo and Wadsö<sup>22</sup>, agree very well with our results for *n*-hexanol and *n*-octanol.

In the next few paragraphs, the values of the free energy, heat capacity, en-

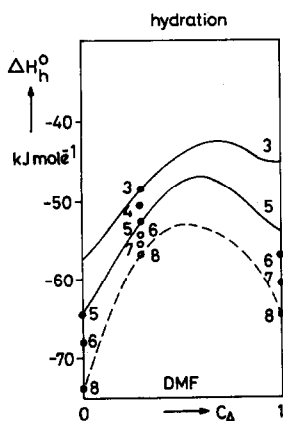


Fig. 5. Standard enthalpies of hydration of *n*-alcohols in DMF–water mixtures. Numbers in the figure denote the value of *n*. Open symbols are our data and closed symbols and full lines are recalculated data from Rouw and Somsen<sup>3</sup>, on enthalpies of solution,  $\Delta H_{sol}^{\circ}$ . Note that  $\Delta H_h^{\circ} = \Delta H_{sol}^{\circ} - \Delta H_{vap}^{\circ}$ , where  $\Delta H_{vap}^{\circ}$  denotes the standard enthalpy of vaporization (from ref. 20).

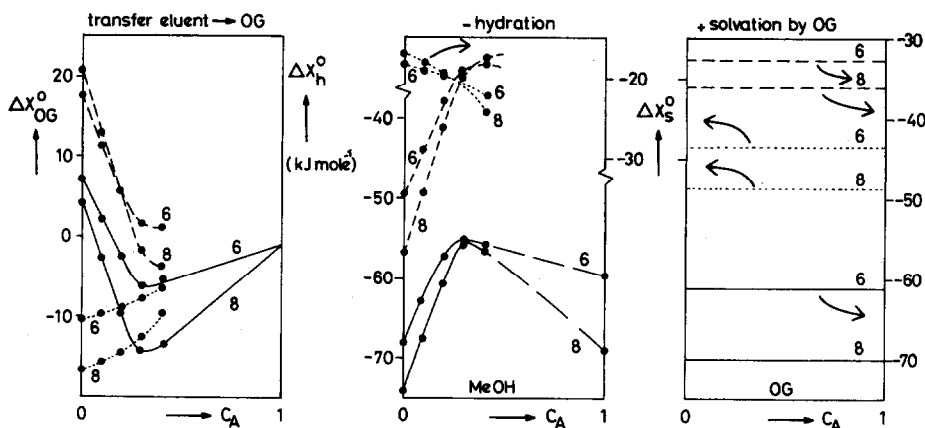


Fig. 6. Thermodynamics of transfer, solvation and hydration in methanol (MeOH)-water mixtures. Symbols as in Fig. 4. Solutes are *n*-hexanol and *n*-octanol (denoted with the value of *n* in the figure). Values were calculated with the  $\Delta X$  equivalent of eqn. 8.  $\Delta G_h^o$  and  $\Delta H_{hn}^o$  values at  $C_A = 0$  are from refs. 20 and 23; values at  $C_A = 1$  are estimated by extrapolation with constant methylene increments from data in ref. 21.

thalpy and entropy will be discussed and interpreted according to current theories on the subject.

### Free energy values

It can be seen in Figs. 6 and 7 that values of  $\Delta G_{OG}^o$  become less negative with increasing amounts of co-solvent, in this case just as with the values of  $-\log K_{X,B}$  discussed before<sup>1</sup>. The ratio of  $\Delta G_{OG}^o$  and  $\Delta G_{OG,CH_2}^o$  (Fig. 4) appears to have a nearly constant value of  $(n - 2.8)$  in all eluents except for methanol ( $C_A = 0.414$ ). Assuming constant methylene increments, this can be interpreted as a contribution of the polar head to  $\Delta G_{OG}^o$  opposite to that of 2.8 methylene groups. This contribution thus decreases from 9 kJ mol<sup>-1</sup> in water to *ca.* 6 in methanol ( $C_A = 0.303$ ). The value for

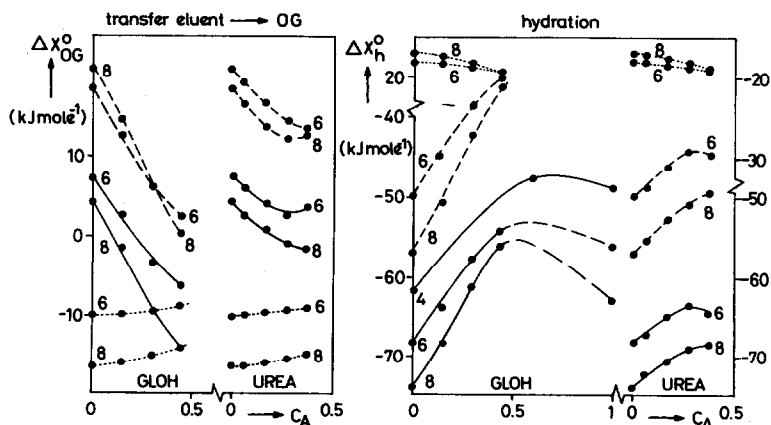


Fig. 7. Thermodynamics of transfer and hydration in eluents with ethylene glycol (GLOH) and urea. Symbols as in Fig. 4. With ethylene glycol, values for *n*-butanol were obtained from ref. 22. Values at  $C_A = 1$  are estimated by extrapolation of values for lower homologues from ref. 24.

the free energy of dehydration of the polar head in water is much larger; *i.e.* *ca.* 24 kJ mol<sup>-1</sup><sup>25</sup>. Appreciable hydrogen-bond formation of the polar head in the layer of OG must therefore still persist, presumably with the spacer arm<sup>8,26</sup>.

The methylene increments of  $\Delta G_{\text{h}}^{\circ}$  and  $\Delta G_{\text{h}}^{\circ}$  are equal to half the difference between the curves of *n*-hexanol and *n*-octanol for solvation and hydration, respectively, in Figs. 6 and 7. The methylene increments of  $\Delta G_{\text{h}}^{\circ}$  are large and negative, *i.e.* -2.7 kJ mol<sup>-1</sup>. This is also observed with solvation in organic liquids<sup>27,28</sup>. In water, the methylene increments of  $\Delta G_{\text{h}}^{\circ}$  for *n* = 6-9 are small and positive, *i.e.* *ca.* +0.5 kJ mol<sup>-1</sup>, thus contributing negatively to  $\Delta G_{\text{OG,CH}_2}^{\circ}$ . The large negative value of  $\Delta G_{\text{OG,CH}_2}^{\circ}$  apparently results from a lack of interaction between water and methylene groups. By addition of co-solvents,  $\Delta G_{\text{h,CH}_2}^{\circ}$  may change sign, as can be seen in Fig. 6. This reflects the increasing capability of the mixture to dissolve hydrophobic moieties. Water is not unique in showing positive values of  $\Delta G_{\text{h,CH}_2}^{\circ}$ . It can be inferred that solvation in hydrazine is also accompanied with small (positive) methylene increments<sup>29</sup>. The cause of this phenomenon lies probably in the large forces of cohesion in polar liquids as a result of extensive hydrogen-bond networks<sup>30</sup>.

#### Heat capacity values

The heat capacity values of *n*-octanol (Fig. 3) change by *ca.* -0.35 kJ mol<sup>-1</sup> K<sup>-1</sup> upon transfer from water to the layer of OG. The large negative value of  $\Delta C_{\text{p,OG}}^{\circ}$  water is in good agreement with the concept of a thermally labile hydrophobic hydration structure, that is thought to surround alkyl groups in water. It is seen that  $\Delta C_{\text{p,OG}}^{\circ}$  becomes less negative by the addition of co-solvent.

Fig. 3 offers the same picture as can be obtained from literature data for the dissolution of *n*-butanol in aqueous mixtures of DMF<sup>7</sup> or ethylene glycol<sup>22</sup>.

#### Enthalpy and entropy: general survey

The influence of co-solvents on these quantities is much more complicated than on free energies or heat capacities. Fig. 4, 6 and 7 yield the following four points of information.

- (1) All co-solvents have the same effect on  $\Delta H_{\text{OG}}^{\circ}$  and  $T\Delta S_{\text{OG}}^{\circ}$ , both for alcohols and methylene groups: their values are lowered. The entropy decreases more rapidly than the enthalpy. A change to negative values may even occur. Note that  $\Delta H_{\text{OG,CH}_2}^{\circ}$  in water is strongly negative. So this type of hydrophobic interaction is not solely entropy-driven, and it becomes less important with increasing temperature.
- (2) The co-solvents are not equally effective in lowering values of  $\Delta X_{\text{OG}}^{\circ}$ . Of course, this depends also on the concentration unit used.
- (3) Changes in  $\Delta G_{\text{OG}}^{\circ}$  can be accompanied by tenfold changes in  $T\Delta S_{\text{OG}}^{\circ}$ . Apparently, changes in entropy and enthalpy largely compensate each other.
- (4) A considerable contribution of the polar head to values of  $\Delta H_{\text{OG}}^{\circ}$  and  $T\Delta S_{\text{OG}}^{\circ}$  must exist. This follows from the positive values of  $\Delta H_{\text{OG}}^{\circ}$  and the negative value of  $\Delta H_{\text{OG,CH}_2}^{\circ}$ . The possibility of non-linear methylene increments over the range *n* = 0-9 hampers the precise evaluation of the contribution of the polar head to  $\Delta H^{\circ}$  and to  $T\Delta S_{\text{OG}}^{\circ}$ . However, a tentative extrapolation to *n* = 0 yields an enthalpic contribution of +16 kJ mol<sup>-1</sup>, and an entropic contribution of +7 kJ mol<sup>-1</sup> to the values of  $\Delta H_{\text{OG}}^{\circ}$  and  $T\Delta S_{\text{OG}}^{\circ}$ , respectively. It follows that the partial dehydration of the polar head is the cause of the increase of retention with increasing temperature

in water (see Fig. 2), and not the hydrophobic interaction of the alkyl chain with the layer of OG.

We shall now turn to the interpretation of the values of  $\Delta H^\circ$  and  $T\Delta S^\circ$  separately.

### Enthalpy values

It can be seen in Fig. 6 that the values of  $\Delta H_h^{\circ*}$  and  $\Delta H_h^\circ$  are nearly equal, in spite of  $\Delta G_h^{\circ*}$  being much less negative (*ca.* 15 kJ mol<sup>-1</sup> for *n*-octanol). This unexpected feature (for it costs a lot of heat energy to form a cavity in the hydrogen-bonded liquid) has been interpreted as the result of an extensive formation of hydrogen bonds around alkyl chains in aqueous solution. The nature of this labile, non-rigid "hydrophobic hydration" and its existence in (partly) aqueous media has been studied by various methods (see *e.g.* refs. 3 and 7, and refs. therein). Water is unique in this respect, for even hydrazine, about as lipophobic as water and also strongly hydrogen-bonded, does not show such abnormal enthalpies<sup>29</sup>.

It has been found that hydrophobic hydration is strongly co-operative in nature. This explains the strikingly non-linear graphs of  $\Delta H_h^\circ$  and  $\Delta H_{OG}^\circ$  as a function of  $C_A$  in the Figs. 6 and 7. Apparently, relatively small amounts of co-solvent are capable of destroying a large portion of the hydrogen bonds around alkyl chains, so that for  $C_A$  *ca.* 0.3 the hydrophobic hydration of octanol is completely nullified. This is also reflected in Fig. 3 for the values of  $\Delta C_{p,OG}^\circ$ .

Following Rouw and Somsen, we adopted a simple model, developed by Mastrianni *et al.*<sup>31</sup> and based on the assumption that hydrophobic hydration causes a contribution  $\Delta H_{hh}^\circ$  in pure water, superimposed on the value obtained from a linear relation in the co-solvent-rich region between  $\Delta H^\circ$  and the mole fraction of water,  $X_{H_2O}$ . This value (in our case  $\Delta H_h^{\circ*} - \Delta H_{hh}^\circ$ ) is obtained by extrapolation to  $X_{H_2O} = 1$  of the supposedly linear part of the  $\Delta H^\circ$  vs.  $X_{H_2O}$  relationship at  $X_{H_2O} < ca. 0.6$ . It is then assumed that the probability of finding  $N$  water molecules forming a "cage" around *e.g.* an alkyl group is equal to  $X_{H_2O}^N$ , and that even the exclusion of only one water molecule from the cage by co-solvent molecules leads to the nullification of the contribution of that cage to the value of  $\Delta H^\circ$  (in our case,  $\Delta H_h^\circ$ ). For solvation in a partly aqueous eluent it can be derived that

$$\Delta H_h^\circ = X_{H_2O}(\Delta H_h^{\circ*} - \Delta H_{hh}^\circ) + (1 - X_{H_2O})\Delta H_{h,C_A=1}^\circ + X_{H_2O}^N \Delta H_{hh}^\circ \quad (10)$$

where  $\Delta H_{h,C_A=1}^\circ$  denotes the standard solvation enthalpy in the pure co-solvent A.

We applied this expression to our data on  $\Delta H_h^\circ$  by adopting successively several values of  $\Delta H_{hh}^\circ$  for the *n*-alcohols and then calculating the best value of  $N$  by the least-squares method. The curves were forced through the  $\Delta H_h^\circ$  values at  $X_{H_2O} = 1$  and 0. It appears that good fits can be obtained only with different values of  $N$  for the various co-solvents (except ethylene glycol and methanol). This is shown in Fig. 8 for *n*-octanol, taking  $\Delta H_{hh}^\circ = -24$  kJ mol<sup>-1</sup>.

Fig. 9 is a plot of the calculated best values of  $N$  as a function of the adopted values of  $\Delta H_{hh}^\circ$  for the *n*-alcohols with  $n = 6, 7$  and 8. The numbers in the graph denote the standard errors of the estimate of  $\Delta H_h^\circ$ ,  $S_e$  (kJ mol<sup>-1</sup>) data for  $X_{H_2O} = 0$  and 1 excluded. It can be seen in Fig. 9 that our data can be fitted with eqn. 10 with  $S_e$  values of *ca.* 1 kJ mol<sup>-1</sup> over a range of  $\Delta H_{hh}^\circ$  values, which hampers the

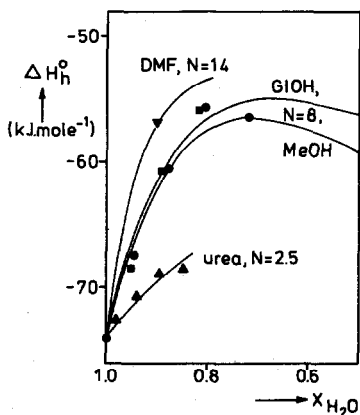


Fig. 8. Enthalpies of hydration of *n*-octanol and the cage model. Symbols as in Fig. 4. Lines are calculated values of  $\Delta H_{hh}^0$ , with a  $\Delta H_{hh}^0$  value of  $-24 \text{ kJ mol}^{-1}$ . The best values of  $N$  are indicated. With urea, the value of  $C_A = 1$  (not shown) is assumed to be equal to that of ethylene glycol. However, even a change of  $10 \text{ kJ mol}^{-1}$  in this value does not lead to different conclusions.

extraction of both  $\Delta H_{hh}^0$  and  $N$  values. With DMF, the values of  $N$  are plotted that reproduce the single experimental  $\Delta H_{hh}^0$  value for *n*-octanol. For the sake of comparison, values of  $N$  and of  $\Delta H_{hh}^0$  for lower homologue (symbol: ●,  $n$ -values in parentheses) obtained by Rouw and Somsen<sup>3</sup> with DMF–water mixtures also are shown. Extrapolation of these data to the curve for *n*-octanol in DMF gives the combination  $\Delta H_{hh}^0 = -24 \text{ kJ mol}^{-1}$ ,  $N = 14$ . With this value of  $\Delta H_{hh}^0$  for *n*-octanol, a lower value for  $N$  is found in methanol–water and ethylene glycol–water mixtures, viz.,  $N = 8$ . With *n*-hexanol and *n*-heptanol,  $N$  values of 6 and 7 are found, respectively, if  $\Delta H_{hh}^0$  values are adopted in between those for  $n = 5$  and 8. On doing so,  $N$  is found to increase with  $n$ , as could be expected.

However, quite independent of the  $\Delta H_{hh}^0$  values are the values of  $N = 2\text{--}3$  found with urea (Fig. 9). They are very much lower than those for the other cosolvents. The low  $N$  values are related to the low value of the slope of the curve for urea in Fig. 8. Apparently, urea does not destroy the cage-like hydration structures

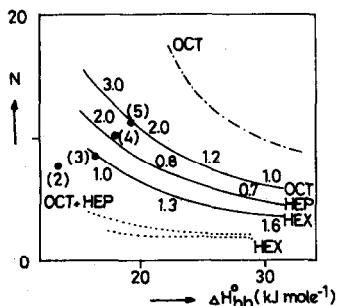


Fig. 9. Best values of  $N$  as a function of adopted values of  $\Delta H_{hh}^0$ . Numbers in the graph denote the standard deviation of estimates of  $\Delta H_{hh}^0$ . The symbol ● ( $n$ ) denotes a value from Rouw and Somsen<sup>3</sup>;  $n$  is the number of carbon atoms of the *n*-alcohol.



as effectively as DMF, for example. Rather, our results point to the existence of mixed solvation structures around alkyl groups in concentrated solutions of urea, as proposed by Nozaki and Tanford<sup>32</sup>. Recently, Kuharski and Rossky<sup>33,34</sup>, in a molecular dynamics study, simulated the incorporation of urea in the hydration layer of an apolar solute. These authors showed that interactions between water–water and water–urea molecules in contact with the solute are indeed somewhat stronger than the same interactions in the bulk solution, although this enhancement was found to be smaller than in pure water. These findings explain our results: urea may be built-in into the hydrophobic hydration structure. However, it should be noted that the values of  $\Delta C_{p,OG}^{\circ}$  in urea–water mixtures indicate even less structure around the alkyl chains than in mixtures of the other co-solvents with water.

Literature values of enthalpies of solution may also be used to calculate  $N$  values. We arrived at  $N = 6$  for  $n$ -butanol in ethylene glycol–water mixtures<sup>22</sup>. This is lower than its value in DMF–water mixtures<sup>3</sup> ( $N = 10$ ), just as we have found for  $n = 6, 7$  and  $8$ . Such dependence of  $N$  values on the size and/or the polarity of the co-solvent molecules is also indirectly apparent in results of Kimura *et al.*<sup>34</sup> with alcohols as co-solvents. Here, the slopes of the enthalpy graph at  $X_{H_2O} = 1$  are different, just as in our Fig. 8. This may indicate that the polar hydroxy group of the co-solvent can also be (partially) built-in into the hydration structure without destroying it, just as the hydroxy group of the solute probably participates in the cage structure<sup>7,35</sup>; it also may explain the lower  $N$  values we found for methanol and ethylene glycol as co-solvents.

#### Entropy values

Figs. 4, 6 and 7 show the same trends (in a somewhat more pronounced way) as enthalpy values. The large, negative values of  $T\Delta S_R^{\circ}$  are thought to arise from the increase of structure, the slowing down of translation and rotation, and the restriction of the configurational freedom of water in the hydration shell associated with hydrophobic hydration<sup>36</sup>. Enthalpy and entropy values compensate at least partially.

#### CONCLUSIONS

(1) Values of  $\Delta G^{\circ}$ ,  $\Delta H^{\circ}$ ,  $\Delta S^{\circ}$  and  $\Delta C_p^{\circ}$  for the interaction of  $n$ -alcohols with octyl-Sepharose can be interpreted with a simple partition model. Their variation with eluent composition is solely caused by the change of the standard state of the solute in the aqueous phase.

(2) The increase of the retention of the  $n$ -alcohols with temperature, with water as the eluent, is not caused by hydrophobic interaction but by dehydration of the polar head.

(3) By adopting the cage model of Mastroianni *et al.*<sup>31</sup>, reasonable values of the number of water molecules  $N$  in the hydration cage of  $n$ -alcohols can be obtained from the enthalpy of hydration. The (single) value for the enthalpy of hydration of  $n$ -octanol in DMF–water can be described with  $N = 14$ . This figure corresponds with the value extrapolated from data given by Rouw and Somsen for lower homologues. With aqueous mixtures of methanol or ethylene glycol, lower  $N$  values are found, *viz.* 8, 7 and 6 for  $n$ -octanol,  $n$ -heptanol and  $n$ -hexanol, respectively.

(4) With urea, much lower  $N$  values indicate that co-solvent molecules are

built-in into the cage. This is not reflected in the values of  $\Delta C_p^0$  in urea-water mixtures. These indicate even less structures around the alkyl chain than in mixtures of the other co-solvents with water.

## REFERENCES

- 1 P. M. Brandts, W. J. Gelsema and C. L. de Ligny, *J. Chromatogr.*, 333 (1985) 41.
- 2 P. M. Brandts, W. J. Gelsema and C. L. de Ligny, *J. Chromatogr.*, 322 (1985) 399.
- 3 A. C. Rouw and G. Somsen, *J. Chem. Thermodyn.*, 13 (1981) 67.
- 4 R. Srinivasan and E. Ruckenstein, *Sep. Purif. Methods*, 9(2) (1980) 267.
- 5 S. Hjertén, in N. Catsimpoalas (Editor), *Methods of Protein Separation*, Vol. 2, Plenum Press, New York, 1976.
- 6 W. H. Scouten, in *Chemical Analysis; a series of monographs on Anal. Chem. and its Applications*, vol. 59, Wiley, 1981, Ch. 9.
- 7 A. C. Rouw, *Thesis*, Vrije Universiteit, Amsterdam, 1982.
- 8 W. J. Gelsema, P. M. Brandts, C. L. de Ligny, A. G. M. Theeuwes and A. M. P. Roozen, *J. Chromatogr.*, 295 (1984) 13.
- 9 A. Ben-Naim, *Hydrophobic Interactions*, Plenum Press, New York, 1980.
- 10 J. C. van Miltenburg and W. E. Hammers, *J. Chromatogr.*, 268 (1983) 147.
- 11 W. E. Hammers and P. B. A. Verschoor, *J. Chromatogr.*, 282 (1983) 41.
- 12 R. Collander, *Acta Chem. Scand.*, 5 (1951) 774.
- 13 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977, p. 96.
- 14 R. Collander, *Acta Chem. Scand.*, 3 (1949) 717.
- 15 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977, p. 166.
- 16 V. Ulbrich, J. Makeš and M. Jureček, *Coll. Czech. Chem. Commun.*, 29 (1964) 1466.
- 17 M. Hirata, S. Ohe and K. Nagahama, *Computer-Aided Data Book of Vapour-Liquid Equilibria*, Kodansha Ltd., Tokyo, 1975.
- 18 *Landolt-Börnstein, Zahlenwerte und Funktionen*, Neue Serie Band 3, Gleichgewichte Siedender Gemische, Springer Verlag, Berlin, 1975.
- 19 *Landolt-Börnstein, Zahlenwerte und Funktionen*, Band II, 1. Teil, Springer Verlag, Berlin, 1971.
- 20 M. H. Abraham, *J. Chem. Soc., Faraday Trans. 1*, 80 (1984) 153.
- 21 C. V. Krishnan and H. L. Friedman, *J. Phys. Chem.*, 75 (1971) 3598.
- 22 S. Nwankwo and I. Wadsö, *J. Chem. Thermodyn.*, 12 (1980) 1167.
- 23 S. Cabani, P. Gianni, V. Mollica and L. Lepori, *J. Solution Chem.*, 10 (1981) 563.
- 24 S. Nwankwo and I. Wadsö, *J. Chem. Thermodyn.*, 12 (1980) 881.
- 25 F. A. Villalonga, R. J. Koftan and J. P. O'Connell, *J. Colloid Interface Sci.*, 90 (1982) 539.
- 26 S. Hjertén, J. Rosengren and S. Pählman, *J. Chromatogr.*, 101 (1974) 281.
- 27 W. Riebesehl, E. Tomlinson and H. J. M. Grünbauer, *J. Phys. Chem.*, 88 (1984) 4775.
- 28 A. Ben-Naim and Y. Marcus, *J. Chem. Phys.*, 81 (1984) 2016.
- 29 M. Sh. Ramadan, D. Fennell Evans and R. Lumry, *J. Phys. Chem.*, 87 (1983) 4538.
- 30 J. H. Hildebrand, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 194.
- 31 M. J. Mastroianni, M. J. Pikal and S. Lindenbaum, *J. Phys. Chem.*, 76 (1972) 3050.
- 32 R. A. Kuharski and P. J. Rossky, *J. Am. Chem. Soc.*, 106 (1984) 5786.
- 33 R. A. Kuharski and P. J. Rossky, *J. Am. Chem. Soc.*, 106 (1984) 5794.
- 34 F. Kimura, S. Murakami and R. Fujishiro, *J. Solution Chem.*, 4 (1975) 241.
- 35 S. Okazaki, K. Nakanishi and H. Touhara, *J. Chem. Phys.*, 78 (1983) 454.
- 36 K. Hallenga, J. R. Grigera and H. J. C. Berendsen, *J. Phys. Chem.*, 84 (1980) 2381.