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ENTHALPIES OF DILUTION OF BIFUNCTIONAL ALCOHOLS IN CONCENTRATED AQUEOUS SOLUTIONS OF UREA AT 298.15 K *

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ABSTRACT

The enthalpies of dilution of aliphatic bifunctional alcohols were measured in 7 m urea aqueous solutions at 298.15 K by flow microcalorimetry. The excess enthalpies were expressed as a power expansion series in the molality of the alcohol, referred to 1 kg of the mixed urea–water solvent.

The values of the second and the third coefficients were all found to be positive and lower than the corresponding values in water. They show an approximately linear dependence on the square of the number of methylene groups. This fact suggests that in the presence of a large amount of urea excess enthalpies are determined primarily by methylene–methylene interactions. This is quite surprising and of remarkable interest on account of its obvious biological implications, since a kind of "lipophobic" or some modified hydrophobic effect seems still to be effective in concentrated urea solutions.

INTRODUCTION

Concentrated aqueous solutions of urea are frequently used as a denaturing medium for proteins, polypeptides and other biopolymers. In the case of globular proteins, in particular, many working hypotheses have been formulated concerning the action of urea on the hydrophobic interactions generally assumed to be one of the main driving forces stabilizing the native conformations [1]. From the increasing solubility of lighter hydrocarbons in the presence of concentrated aqueous urea with respect to pure water at room temperature [2], it has usually been inferred that urea weakens the hydrophobic interactions. The same conclusion was reached in studies on the thermodynamic transfer properties of solutes containing hydrophobic groups, from water to aqueous urea solutions [3,4]. However, comparison of

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the solubilities of methane and ethane as a function of the concentration of urea and temperature led Ben Naim and Yaacobi [5] to conclude, on the basis of their statistical-mechanical model, that hydrophobic interactions are strengthened by the presence of urea, at least for small alkyl groups.

For these reasons, we are carrying out a joint research programme on the physical chemistry of concentrated aqueous solutions of urea containing small molecules of biological interest, such as amides, protected amino acids, alcohols and sugars [6–9]. A calorimetric study of the interactions between bifunctional alcohols in concentrated aqueous solutions of urea is reported here. Mono- and bifunctional alcohols also affect the conformational stability of proteins but with a mechanism different from that of urea, which depends on the hydrophobicity of each alcohol. However, in the present study they are primarily considered as "soluble hydrocarbons".

THERMODYNAMICS

The excess enthalpy of binary solutions (assuming a reference state of infinite dilution for the solute, and a pure liquid—a binary mixture in this study—as the reference state for the solvent) can be defined as follows

$$H^{\mathrm{E}}(m) = H(m) - H_1^0 - m\overline{H}_x^0 = G^{\mathrm{E}}(m) - TS^{\mathrm{E}}(m) \tag{1}$$

where H^{E} and H are the excess and total enthalpies of the system and H_1^0 is that of the solvent, all referred to 1 kg of solvent. $\overline{H}_x^0$ is the limiting partial molar enthalpy of the solute. $G^{\mathrm{E}}(m)$ and $S^{\mathrm{E}}(m)$ are the corresponding excess free energy and entropy respectively. If the urea–water mixture is regarded as being a mixed solvent, eqn. (1) retains its general validity.

According to the McMillan–Mayer theory of solutions, as adapted by Kauzmann and Friedman to non-electrolyte solutions [10–12], the excess thermodynamic properties can be expanded as a power series in the molality of the solute, e.g.

$$H^{\mathrm{E}}(m) = h_{xx}m^2 + h_{xxx}m^3 + \ldots \tag{2}$$

where each coefficient is the enthalpic contribution to the virial coefficients of the Gibbs excess energy $G^{\mathrm{E}}(m)$

$$G^{\mathrm{E}}(m) = g_{xx}m^2 + g_{xxx}m^3 + \ldots \tag{3}$$

The coefficient g_{xx} is, in turn, related to the second coefficient of the virial expansion of the osmotic pressure $B_{xx}^{\star}$, which has a clear statistical-mechanical significance

$$B_{xx}^{\star} = -\tfrac{1}{2}\int_0^{\infty} \langle \{\exp[-W(r, \phi_i)/kT] - 1\}\rangle 4\pi r^2 \,\mathrm{d}r \tag{4}$$

where $W(r, \phi_i)$ is the mean force potential between two solute molecules. This is a function of the separation distance r and of a set of angles ϕ_i which define the reciprocal orientation of the two solute molecules. The quantity between the broken brackets ⟨ ⟩ needs, in the statistical calculations, to be averaged on all the possible orientations of the solvent molecules. In this manner $B^{\star}_{xx}$, g_{xx}, h_{xx}, etc. account implicitly for the effects owing to rearrangement in solute–solvent and solvent–solvent interactions with respect to infinite dilution. The McMillan–Mayer formalism is thus also useful in describing the properties of solutions in mixed solvents. However, it must be remembered that the solvation processes are of a dual nature and in competition with each other, and that the mean force potential will depend on the orientations of two different kinds of solvent molecules.

EXPERIMENTAL

The enthalpies of dilution of a constant 7 m urea concentration in water $\Delta_{\mathrm{dil}}H$ (in J kg^{-1} of mixed solvent in the final solution) were determined using an LKB 2107 standard flow microcalorimeter at 298.15 ± 0.02 K. Details of the experimental procedure have been described elsewhere [13–16]. Diols (99% Aldrich products) were purified according to the literature [15]. Urea was recrystallized from ethanol and vacuum dried at 45 °C. Solutions were prepared by weight before each set of measurements. The excess enthalpies obtained were expressed as a power expansion series in the molality of the diol, referred to 1 kg of the mixed urea–water solvent.

RESULTS

The experimental heats of dilution are reported in Table 1 along with the initial and final molalities (calculated on the basis of 1 kg of mixed solvent). The abbreviations used for the bifunctional alcohols (x) are: ED, ethanediol; 1,2-PD, 1,2-propanediol; 1,3-PD, 1,3-propanediol; 1,2-BD, 1,2-butanediol; 1,3-BD, 1,3-butanediol; 1,4-BD, 1,4-butanediol; 2,3-BD, 2,3-butanediol; 1,5-PeD, 1,5-pentanediol; and 1,6-HxD, 1,6-hexanediol. The second virial coefficients are given in Table 2 and are compared with the corresponding values obtained in water [15–18]. They are the enthalpic parts of the pairwise virial coefficients of the excess Gibbs energies. All the values are lower in the case of the mixed solvent, but the differences $\Delta h(\mathrm{W} \rightarrow \mathrm{U})$, last columns of Table 2, are relatively small. The h_{xx} values are thus still positive, of the same order of magnitude as those in water, and increase when the number of methylene groups is increased. This is quite surprising because it is usually assumed that urea destroys the hydrophobic interactions. The third coefficients reported in Table 2 also show that the differences from the corresponding quantities in water are very small.

TABLE 1

Enthalpies of dilution for various aliphatic diols (x) in 7 m aqueous urea solution at 298.15 K

m_i [a]	m_f [a]	$-\Delta_\mathrm{dil}H/m_\mathrm{f}$ [b]	$\Delta_\mathrm{dil}H/m_\mathrm{f}(m_\mathrm{f}-m_\mathrm{i})$ [c]	$m_\mathrm{f}+m_\mathrm{i}$ [a]
x = ED				
0.3661	0.1839	54.6	300	0.5500
0.3777	0.1839	57.8	298	0.5616
0.4088	0.1919	73.7	288	0.6008
0.5383	0.2616	77.5	280	0.8000
0.6842	0.3338	97.8	279	1.0180
0.7537	0.3662	107	275	1.1200
0.8627	0.4189	119	268	1.2816
0.9100	0.4417	124	265	1.3518
0.9807	0.4758	132	262	1.4566
1.2751	0.6197	160	245	1.8948
x = 1,2-PD				
0.1428	0.0687	32.3	430	0.2115
0.1350	0.0650	30.4	435	0.2000
0.2704	0.1301	61.7	440	0.4005
0.3252	0.1564	74.6	442	0.4816
0.4052	0.1949	94.6	450	0.6001
0.4483	0.2114	106	447	0.6598
0.4840	0.2359	109	440	0.7200
0.5486	0.2694	126	450	0.8180
0.6187	0.3028	142	453	0.9195
0.8292	0.3987	199	463	1.2279
0.8644	0.4156	206	460	1.2800
x = 1,3-PD				
0.3666	0.1734	64.7	335	0.5400
0.4467	0.2128	79.1	338	0.6595
0.4729	0.2236	84.5	339	0.6965
0.5370	0.2629	94.6	345	0.8000
0.5852	0.2866	102	343	0.8718
0.6614	0.3185	118	345	0.9799
0.8323	0.4069	149	351	1.2392
0.9292	0.4542	166	350	1.3784
1.2134	0.5866	228	365	1.8000
x = 1,4-BD				
0.21540	0.1086	55.5	520	0.3240
0.3253	0.168	82.2	525	0.4941
0.4833	0.2572	121	535	0.7405
0.5221	0.2788	131	535	0.8000
0.6682	0.3467	174	540	1.0115
0.8103	0.4206	212	541	1.2310
1.0085	0.5145	271	549	1.5230
1.0991	0.5594	299	555	1.6585

TABLE 1 (continued)

m_i [a]	m_f [a]	$-\Delta_{dil}H/m_f$ [b]	$\Delta_{dil}H/m_f(m_f-m_i)$ [c]	m_f+m_i [a]
$x=$1,3-BD				
0.1660	0.0850	35.7	441	0.2510
0.1850	0.0164	74.3	441	0.2768
0.1986	0.1013	42.3	435	0.3005
0.2727	0.1391	57.8	433	0.4118
0.3649	0.1861	78.6	440	0.5510
0.4979	0.2539	107	438	0.7518
0.6762	0.3448	144	436	1.0210
0.7589	0.3281	187	435	1.0870
$x=$1,2-BD				
0.1347	0.0652	53.0	765	0.2000
0.1402	0.0715	52.8	770	0.2118
0.1662	0.0848	62.7	771	0.2510
0.1902	0.0971	71.9	772	0.2873
0.2654	0.1354	101	780	0.4008
0.3049	0.1483	122	780	0.5432
0.3388	0.1728	128	769	0.5116
0.4044	0.1961	162	780	0.6005
0.4975	0.2537	189	776	0.7513
0.5298	0.2701	202	779	0.8000
0.6628	0.3188	270	781	0.9817
$x=$2,3-BD				
0.1351	0.0653	40.5	580	0.2005
0.1685	0.0822	50.4	585	0.2508
0.2701	0.1305	81.6	585	0.4006
0.2788	0.1404	94.5	588	0.4272
0.3146	0.1539	86.9	590	0.4680
0.4106	0.2004	126	600	0.6110
0.5496	0.2642	169	591	0.8138
0.5510	0.2689	166	590	0.8200
0.7056	0.3443	218	605	1.0500
0.7802	0.3767	246	610	1.1569
0.7930	0.3870	252	620	1.1800
0.9751	0.4758	304	609	1.4510
$x=$1,5-PeD				
0.1073	0.0533	40.5	751	0.1605
0.1343	0.0667	50.4	745	0.2010
0.2282	0.1073	92.9	769	0.3375
0.3208	0.1592	124	770	0.4800
0.3669	0.1775	150	780	0.5474
0.4122	0.1978	165	771	0.6100
0.4684	0.2250	190	780	0.6934
0.5405	0.2595	220	785	0.8000
0.5690	0.2760	233	798	0.8450
0.5800	0.2812	239	800	0.8612
0.6400	0.3049	265	790	0.9449

TABLE 1 (continued)

m_i [a]	m_f [a]	$-\Delta_{dil}H/m_f$ [b]	$\Delta_{dil}H/m_f(m_f-m_i)$ [c]	m_f+m_i [a]
x = 1,6-HxD				
0.0600	0.0228	38.7	1041	0.0828
0.0681	0.0324	38.3	1030	0.1005
0.1287	0.0608	37.5	1050	0.1895
0.1500	0.0698	71.9	1050	0.2198
0.2741	0.1308	158	1100	0.4049
0.3660	0.1740	223	1160	0.5400
0.3984	0.1938	225	1099	0.5922
0.5000	0.2416	284	1100	0.7416
0.5695	0.2705	350	1170	0.8400

[a] Units, mol kg^{-1}. [b] Units, J kg mol^{-1}. [c] Units, J kg mol^{-2}.

The behaviour of the bifunctional alcohols resembles that of the more hydrophobic alkanols, but with some important differences. First of all, the third enthalpic virial coefficients are significant for most of the diols, but only exceptionally for alkanols in concentrated urea [8]. This is an indication that diols are involved in a more complex network of interactions than normal alkanols, even in the mixed solvent.

In Fig. 1, the h_{xx} values are reported for all the diols in aqueous 7 m urea as a function of the square of the number of methylene groups. The solid line represents the best fit for the four more hydrophobic α,ω-diols. The slope of the linear plot (a measure of the methylene–methylene enthalpic contributions to the solute–solute interactions in the particular solvent) has a value of 26 ± 1 J kg mol$^{-2}n_c^{-2}$ (where n_c is the number of methylene groups) for the α,ω-diols, excluding the head of the series, ethanediol. The reported uncertainty is the 95% confidence limit. This value is comparable with that of 35 ± 6 J kg mol$^{-2}n_c^{-2}$ found in a preceding paper by applying the Savage and Wood analysis of the group additivity [19] to the mono- and polyhydric alcohols in water [20]. For alkanols in 7 m urea, a value of 38 ± 4 J kg mol$^{-2}n_c^{-2}$ was found [8]. This is just at the upper limit of the confidence range obtained by analysing the enthalpies of the diluted aqueous solution according to Savage and Wood. Note that the experimental data fit to a line having a value at the intercept of 95 ± 18 J kg mol^{-2} in the case of the set of the four more hydrophobic α,ω-diols. On considering the diols all together, the following values are obtained: 23 ± 7 J kg mol$^{-2}n_c^{-2}$ and 201 ± 138 J kg mol$^{-2}n_c^{-2}$ for the slope and the intercept respectively. Imposition of a nil intercept gives a slope of 32 ± 5 J kg mol$^{-2}n_c^{-2}$ which is a better fit. For the diols, as for the alkanols (and for the more limited sets of the α,ω-diols or $[j, j+1]$-diols), the fitting of expressions such as $h_{xx} = a + bn_c + cn_c^2$ is worse. Analysis of the data must necessarily be confined to this kind of representation because of the difficulties of applying

TABLE 2

Second and third enthalpic coefficients for aliphatic diols in water and 7 m aqueous urea at 298.15 K (95% confidence limits in brackets). The data in water are from refs. 15–18

	h_{xx}(U, 7 m) ($\mathrm{J\ kg\ mol^{-2}}$)	h_{xx}(W) ($\mathrm{J\ kg\ mol^{-2}}$)	$\Delta h_{xx}(\mathrm{W \rightarrow U})$ ($\mathrm{J\ kg\ mol^{-2}}$)	h_{xxx}(U, 7 m) ($\mathrm{J\ kg^2\ mol^{-3}}$)	h_{xxx}(W) ($\mathrm{J\ kg^2\ mol^{-3}}$)	$\Delta h_{xxx}(\mathrm{W \rightarrow U})$ ($\mathrm{J\ kg^2\ mol^{-3}}$)
Ethanediol	315(6)	415(30)	−100	−37(6)	−21(16)	−16
1,2-Propanediol	428(4)	593(13)	−165	26(6)	5(7)	21
1,3-Propanediol	325(4)	523(9)	−198	21(4)	−5(2)	26
1,2-Butanediol	767(5)	923(5)	−156	15(10)	60(3)	−45
1,3-Butanediol	439(5)	750(6)	−311	−4(7)	14(4)	−18
1,4-Butanediol	514(4)	787(1)	−273	25(3)	−9(2)	34
2,3-Butanediol	577(8)	837(10)	−260	27(10)	51(9)	−24
1,5-Pentanediol	741(10)	1335(25)	−594	60(16)	−20(10)	80
1,6-Hexanediol	1026(32)	2402(35)	−1376	154(68)	−40(30)	194

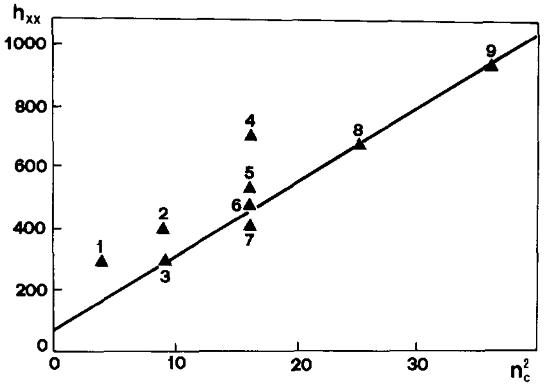

Fig. 1. Second virial coefficients of the excess enthalpies for the diols in 7 m aqueous urea at 298.15 K as a function of the square of the number of methylene groups n_c^2. Units for h_{xx}, J kg mol^{-2}. 1, Ethanediol; 2, 1,2-propanediol; 3, 1,3-propanediol; 4, 1,2-butanediol,; 5, 2,3-butanediol; 6, 1,4-butanediol; 7, 1,3-butanediol; 8, Pentanediol; 9, hexanediol. The straight line fits the points 3, 6, 8 and 9.

any additive approach to a limited number of data. Statistical analyses, such as that of the additivity of group interactions by Savage and Wood [19], cannot always be carried out successfully in detail on this basis, because solutes such as diols present many stereochemical complexities. A direct comparison with the same limited number of aqueous dilute systems is not easy and can prove ambiguous. In water, the four α,ω-diols considered give a value of -243 ± 223 J kg mol^{-2} for the intercept, while for all the diols the intercept values are positive. This is a result of a very large scattering of the data in water, depending on the complexity of these systems. When a sufficiently large set of data is available, it is preferable to use the Savage and Wood method or, at least, refer to this approach.

DISCUSSION

Dilute solutions of urea and α,ω-diols have been studied previously [16]. The results have shown that the attractive urea–diol interaction is also a result of a positive contribution of the entropic component to the pairwise interaction Gibbs energy g_{xy}. This seems to be in line with other results [14,21], indicating the importance of the solvent-mediated effect rather than direct hydrogen bonds in interactions between solutes in water.

If the excess Gibbs energies are unavailable, we cannot make a satisfactory interpretation for systems containing concentrated urea on the basis of calorimetric data alone. However, the preliminary results and their resemblance to those obtained for alkanols [6,8] suggest that a kind of modified hydrophobic effect occurs even in water–urea mixtures at high urea concentrations. The methylene–methylene, solvent-"mediated" or -"assisted" interactions do, in fact, seem still to be an important effect whose enthalpic terms are quantitatively comparable with those found in water. The other interactions, in particular the hydrophilic ones, give zero or positive values (Fig. 1). In reality, the limited number of available data and the experimental uncertainties do not allow a distinction to be made between the contributions of hydrophilic interactions and those from the mixed polar group–apolar group interactions (whose contribution may be assumed to be proportional to the number of methylene groups). The fact that the contribution to the h_{xx} values arising from hydrophilic interactions is positive or around zero in concentrated urea solutions is extremely interesting. As for other polar solutes in the same kind of solvent [6–9,22], it can be envisaged that urea (which is more polar than water and occupies more than 50% of the solution volume at the concentrations used) preferentially solvates hydrophilic groups. Consequently, the water will be forced into the proximity of apolar residues, which will be solvated by water only because the actual geometry of this molecule allows ice-like cages (which can host the alkyl groups) to be built up. Among the polar interactions, the urea–water and urea–urea interactions will also undoubtedly be reinforced in the proximity of domains of low polarizability. However, only water can optimize a network of bonds and interactions, thus allowing an inert molecule or group to be hosted. Moreover, the preferential urea–urea, water–water and urea–water interactions will "push" hydrophobic hydrated chains or groups against each other and release water when the diol concentration increases. We have preferred to call this kind of residual hydrophobic effect a "lipophobic effect" to underline the active role of the mixed solvent [6].

The methylene–methylene interactions seem to be less effective for α,ω-diols than the monofunctional alcohols. This probably results from the dimensions of the urea molecule. One urea molecule can interact with both the hydroxyl groups of a diol molecule or, alternatively, two urea molecules can interact with two hydrophilic groups. In both cases, the presence of the large urea molecule partially inhibits the formation of the water cage around the alkyl groups. For alkanols, this fact may have a less remarkable consequence because only one urea molecule can be involved per alcohol molecule.

Analysis of the $\Delta h_{xx}(\mathrm{W} \rightarrow \mathrm{U})$ values as a function of the equivalent number of methylene groups for the α,ω-diols shows that this quantity diverges rapidly. This trend results from the fact that both the hydrophobic effect in water and the lipophobic effect in the mixture urea–water are predominant (owing to the higher number of methylene–methylene interactions possible), but with different effectiveness, since the dependence on the square of the number of equivalent groups is also reflected in the differential values. If the properties of the butanediols in concentrated urea are considered, Table 1 shows that the order of decreasing values of h_{xx} is 1,2 > 2,3 >

1,4 > 1,3. This series can be correlated with a reasonable decreasing order of exposure of the hydrophobic groups to the solvent, i.e. to the decreasing series of hydrophobic diol–diol interactions. The same behaviour is shown by the two propanediols.

The behaviour of the third coefficients h_{xxx} is also interesting. They change from a negative value (for ethanediol) to increasing positive values on increasing the number of methylene groups, with the exception of 1,3-butanediol. The preferential interactions with urea and water are probably responsible for this behaviour and the trend of the $\Delta h_{xxx}(W \rightarrow U)$ values.

Because the structure of the urea–water mixture is completely changed with respect to that of pure water, it is possible that the positive entropic effects in that particular mixed solvent are not as large as those occurring in water. These entropic contributions, as is well known, overwhelm the positive enthalpic terms in water, making the excess Gibbs energy negative and leading to the characteristic favourable solute–solute interactions, "assisted" by water, which we call the hydrophobic effect. Work is in progress in order to obtain activity or free energy data in concentrated aqueous solutions of urea or other co-solvents.

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