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NMR studies on hydrophobic interactions in solution

Part 2.†—Temperature and urea effect on the self-association of ethanol in water

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The self-association of ethanol has been investigated in binary aqueous mixtures and in mixtures containing urea as a third component by the application of NMR. We obtain the so-called association parameter A_{22} by means of the measurement of intermolecular ^1H – ^1H dipole–dipole relaxation rates and of self-diffusion coefficients of the ethanol molecules. The composition dependence of A_{22} shows a surprisingly high self-association of ethanol. This result is in agreement with that found recently from other authors using different experimental methods. Addition of urea further increases the self-association of ethanol. For the first time we measure the A_{22} parameter as a function of temperature, ranging from 10 to 40 °C. We observe that the association of ethanol increases with increasing temperature in agreement with theoretical predictions. The structure breaking effect of urea obviously acts on the hydrophobic association in the same way as an increase of temperature.

In recent years several papers investigating solute–solute interactions in water have been published to describe better the ‘hydrophobic interaction’ phenomenon whose role is relevant in many biochemical processes.¹ The term hydrophobic interaction is here used to describe the hydrophobic association of solute molecules through their apolar moieties driven by the surrounding water molecules.²

The associative properties of hydrophobic molecules can be studied in detail using a method based on NMR spectroscopy,^{3–5} through the determination of the so-called A_{22} parameter defined as an integral over an expression containing an atom–atom pair correlation function [$g_{22}(r)$]. This A_{22} parameter conveys information on the sharpening or flattening of the atom–atom pair correlation function if the composition of the system under investigation is changed.

Recently, we have studied, in pure and in salt-containing water, the association behaviour of dimethyl sulfoxide, *tert*-butyl alcohol and 1,1,3,3-tetramethylurea,⁶ and propan-1-ol.⁷ In the last case we analysed not only the binary mixture but also the effect on it of the addition of urea, a characteristic non-electrolyte that is well known for its influence on the denaturation of proteins.

In this paper, we focus our attention on the binary system water + ethanol and the ternary system water + ethanol + urea. For the first time, the measurements were done at three different temperatures, *viz.*, 10, 25 and 40 °C, in order to investigate the effect of temperature upon the association behaviour of ethanol. Information about the temperature dependence of hydrophobic association is scarce but interesting conclusions can be drawn from such investigations.

Theory

The A_{22} parameter is related^{3,8} to the sharpness of the pair correlation function $g_{22}(r)$ and thus to the attractive or repulsive interactions between molecules of the same species in the liquid state and is defined as

$$A_{22} = (1/T_1)_{\text{inter}} \cdot D/c \quad (1)$$

where $(1/T_1)_{\text{inter}}$ is the intermolecular nuclear magnetic dipole–dipole relaxation rate of interacting nuclei in the molecules of interest. Usually it is the interaction between ^1H nuclei that is

observed because these nuclei possess a high value of the gyromagnetic ratio, γ , resulting in a comparatively strong dipole–dipole interaction. D represents the translational diffusion coefficient of the molecules, c is the number density (spin cm^{-3}) of the interacting spins.

Neglecting the details in the A parameter’s theoretical description (a more detailed description is given elsewhere^{3,8}) we can affirm that the A_{22} parameter is related to the atom–atom pair correlation function $g_{22}(r)$ through the equation

$$A_{22} = \frac{4\pi K}{3a^4} \int_a^\infty \left(\frac{a}{r}\right)^6 g_{22}(r) r^2 dr \quad (2)$$

where $K = 3/2\gamma_{\text{H}}^4 h^2$ and a is the closest approach distance of interacting nuclei. Eqn. (2) gives the direct link between the measurable quantity A_{22} and the pair correlation function $g_{22}(r)$ of the atoms carrying the interacting spins. For an ideal mixture $g_{22}(r)$ is independent of the concentration and so A_{22} is constant. If there is self-association among the molecules containing the relaxing spins, $g_{22}(r)$ sharpens, and A_{22} increases with decreasing concentration of spins.^{6,8} So, the detection of association tendency is performed by determining A_{22} as a function of the concentration.

According to eqn. (1), in order to determine the A_{22} parameter it is necessary to measure the ^1H intermolecular relaxation rate of protons in the methyl (or methylene) groups of ethanol brought about by the methyl (or methylene) groups of the nearby ethanol molecules. By NMR relaxation measuring techniques we first obtain the total relaxation rate, given by:

$$(1/T_1)_{\text{tot}} = (1/T_1)_{\text{intra}} + (1/T_1)_{\text{inter}} \quad (3)$$

Separation of the $(1/T_1)_{\text{inter}}$ and $(1/T_1)_{\text{intra}}$ contribution is obtained by performing an isotope dilution experiment.^{9,10} The translational diffusion coefficient, D , of ethanol molecules in the systems investigated was determined by using the NMR spin–echo pulsed-gradient technique, and to obtain c we carried out density measurements. In this manner we were able to determine the A_{22} parameter in the whole composition range of water–ethanol mixtures.

Experimental

D_2O + ethan[^2H]ol and D_2O + ethan[^2H]ol + [$^2\text{H}_4$]urea systems measurements were performed and for the latter the urea concentration was kept constant ($x_3 = 0.09$) in the whole

† Part 1: ref. 7.

composition range of the mixtures. From now on the mole fractions of water, ethanol and urea will be indicated with x_1 , x_2 and x_3 , respectively.

We carried out the following experimental measurements: (i) ^1H spin-lattice relaxation times (by the inversion recovery method); (ii) the translational diffusion coefficients, D , of ethan[^2H]ol; and (iii) the densities of all the systems. The ^1H signals were also used in low-resolution spin-echo measurements to determine the diffusion coefficients. We utilized D_2O and [$^2\text{H}_4$]urea to avoid ^1H signal contributions from water and urea. We used fully deuteriated [$^2\text{H}_5$]ethan[^2H]ol for the isotope dilution experiments. These experiments were performed in the following way. At every x_2 value we substituted $\text{C}_2\text{H}_5\text{OD}$ by a fraction of fully deuteriated ethanol [$x(\text{C}_2\text{D}_5\text{OD})$] with $x = 0, 0.25, 0.50$ and 0.75 and measured T_1 values of ^1H of ethan[^2H]ol. The linear dependence of T_1 was extrapolated to $x(\text{C}_2\text{D}_5\text{OD}) = 0$, delivering the $(1/T_1)_{\text{intra}}$ with a standard deviation of better than 1%. Using eqn. (3) $(1/T_1)_{\text{inter}}$ could be derived.

The ^1H spin-lattice relaxation times T_1 were measured under high-resolution conditions at 200 MHz using a Varian XL-200 instrument. The experimental error in the T_1 measurements was $\pm 2\%$, which includes contributions from temperature instabilities. All of the samples were prepared by weighing and were degassed by several freeze-pump-thaw cycles to eliminate paramagnetic oxygen.

A Minispec pc120 instrument coupled with a commercial pulsed field gradient unit, both from Bruker, were utilized for measuring the translational diffusion coefficients of ethan[^2H]ol. For details of the measurements see ref. 11 and 12. The experimental error in the diffusion coefficients was $\pm 2\%$. However, at the lowest ethan[^2H]ol concentration, because of low concentration of protons, we used a high-resolution spectrometer (Tesla FT, 80 MHz) coupled to a commercial pulsed field gradient unit provided by Stelar.

The density measurements were performed with an Anton Paar densimeter.

All measurements were performed at 10, 25 and 40°C . The temperature instability was less than 0.5°C .

The following chemicals were used: (i) D_2O (99.9%, Aldrich), (ii) ethan[^2H]ol (99.5%, Aldrich), (iii) [$^2\text{H}_4$]urea (98%, Aldrich) (iv) [$^2\text{H}_5$]ethan[^2H]ol (99%, Aldrich). All products were used without further purification. The [$^2\text{H}_4$]urea was dried carefully under vacuum before use.

Results and Discussion

Translational diffusion coefficients

In Fig. 1 the translational diffusion coefficients of ethanol in aqueous mixtures, without urea, at the three different temperatures, are shown. The presence of a shallow minimum near $x_2 \approx 0.20$, indicates that the molecular motion of the ethanol molecules reaches a minimum in the water-rich region. A similar minimum at $x_2 \approx 0.2$, is also found for the diffusion coefficients of propan-1-ol and *tert*-butyl alcohol in water,^{6,7} as well as for other quantities of the aqueous solutions of alcohols,¹³ indicating that in this composition range hydrophobic effects play an important role.

In Fig. 2 the D values of ethan[^2H]ol in the ternary system D_2O –ethan[^2H]ol–[$^2\text{H}_4$]urea ($x_3 = 0.09$) at 10, 25 and 40°C in the composition range $0 < x_2 < 0.3$ are reported. It is interesting to note that at 10°C the diffusion coefficients of ethanol in the presence of urea are higher than those found in the binary system without urea, up to $x_2 \approx 0.2$, where a crossing point is found. With rising temperature this crossing point of the two curves shifts towards higher water content. The phenomenon of a solute promoting the mobility of solvent is called the 'structure-breaking' effect and is almost unique for aqueous solutions. Here we observe the increased mobility of

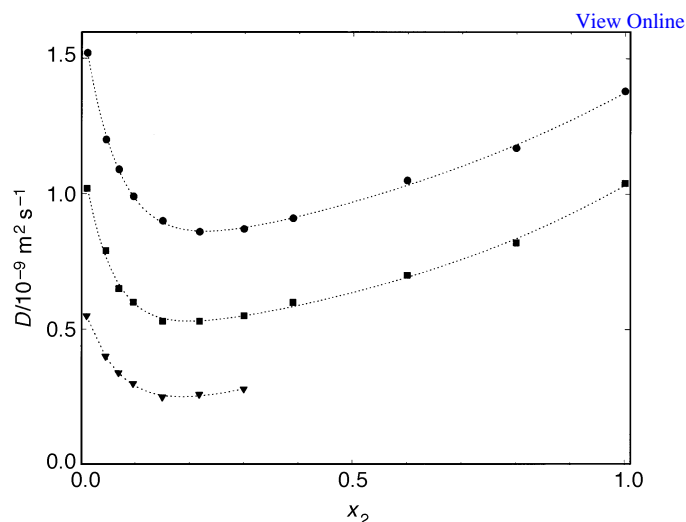


Fig. 1 Self-diffusion coefficients, D , of ethan[^2H]ol in D_2O + ethan[^2H]ol mixtures at 10 (∇), 25 (\blacksquare) and 40°C (\bullet) as a function of the mole fraction of alcohol (x_2)

the second solute, namely ethan[^2H]ol. We assume, as in our previous paper,⁷ that the structure breaking effect caused by [$^2\text{H}_4$]urea is transferred from the water to the ethan[^2H]ol molecules indicating a strong coupling of the molecular motions of both components. Similar effects were found for structure-breaking ions in binary aqueous systems.⁶ The shifting of the crossing point to lower alcohol concentrations with increasing temperature can be ascribed to the change of the structure-breaking ability of [$^2\text{H}_4$]urea, which is stronger at lower temperatures where the water is more structured. We will return to this argument below where the structure-breaking effect is also reflected in the A_{22} parameter results.

A_{22} parameter

In Tables 1–3 the experimental values for $(1/T_1)_{\text{inter}}$ of CH_3 protons are reported. It appears that the intermolecular relax-

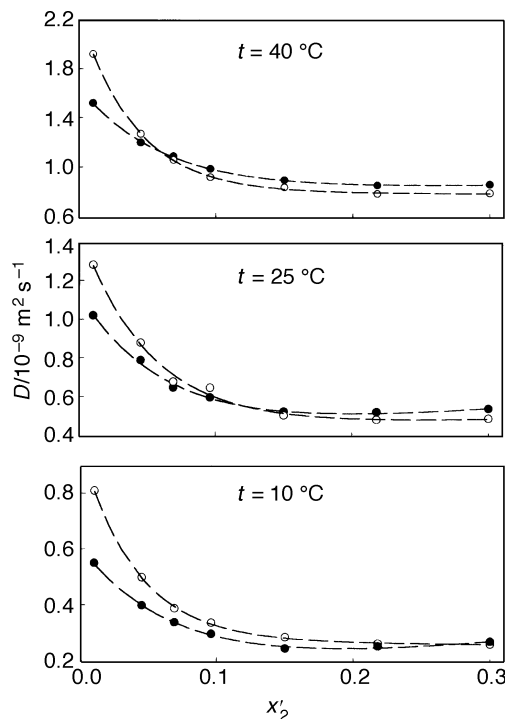


Fig. 2 Self-diffusion coefficients, D , of ethan[^2H]ol in D_2O + ethan[^2H]ol (\bullet) and D_2O + ethan[^2H]ol + [$^2\text{H}_4$]urea (\circ) mixtures at different temperatures as function of $x'_2 = x_2/(x_1 + x_2)$

Table 1 Experimental results used for the determination of A_{22} parameters in the $C_2H_5OD + D_2O$ system at 10 °C

| $X_{C_2H_5OD}$ | $(1/T_1)_{inter}/s^{-1}$ | $c/10^{23} \text{ cm}^{-3}$ | $D/10^{-9} \text{ m}^2 \text{ s}^{-1}$ | $A/10^{-39} \text{ m}^5 \text{ s}^{-2}$ |
|----------------------------------------------|--------------------------|-----------------------------|----------------------------------------|-----------------------------------------|
| $C_2H_5OD + D_2O$ | | | | |
| 0.01 | 0.011 | 0.0164 | 0.55 | 3.7 |
| 0.045 | 0.038 | 0.0687 | 0.40 | 2.2 |
| 0.069 | 0.058 | 0.1018 | 0.34 | 1.9 |
| 0.096 | 0.073 | 0.1351 | 0.30 | 1.6 |
| 0.15 | 0.112 | 0.1921 | 0.25 | 1.5 |
| 0.218 | 0.137 | 0.2547 | 0.26 | 1.4 |
| 0.3 | 0.156 | 0.3124 | 0.28 | 1.4 |
| $C_2H_5OD + D_2O + [^2H_4]urea (x_3 = 0.09)$ | | | | |
| 0.01 | 0.009 | 0.0132 | 0.81 | 5.5 |
| 0.045 | 0.034 | 0.0563 | 0.50 | 3.0 |
| 0.069 | 0.053 | 0.0835 | 0.39 | 2.5 |
| 0.096 | 0.064 | 0.1112 | 0.34 | 2.0 |
| 0.15 | 0.096 | 0.1624 | 0.29 | 1.7 |
| 0.218 | 0.127 | 0.2173 | 0.27 | 1.6 |
| 0.3 | 0.145 | 0.2744 | 0.27 | 1.4 |

ation rates for CH_3 - and CH_2 -protons were, within error limits, equal, although the total relaxation rate for both types of protons differed markedly, as expected. This equality of $(1/T_1)_{inter}$ for different groups of protons has been found previously for propanol.⁷ Values for D and c , needed to calculate the A_{22} parameter for water + ethanol mixtures (both with

Table 2 Experimental results used for the determination of A_{22} parameters in the $C_2H_5OD + D_2O$ system at 25 °C

| $X_{C_2H_5OD}$ | $(1/T_1)_{inter}/s^{-1}$ | $c/10^{23} \text{ cm}^{-3}$ | $D/10^{-9} \text{ m}^2 \text{ s}^{-1}$ | $A/10^{-39} \text{ m}^5 \text{ s}^{-2}$ |
|----------------------------------------------|--------------------------|-----------------------------|----------------------------------------|-----------------------------------------|
| $C_2H_5OD + D_2O$ | | | | |
| 0.01 | 0.007 | 0.0163 | 1.02 | 4.4 |
| 0.045 | 0.026 | 0.0684 | 0.79 | 2.9 |
| 0.069 | 0.034 | 0.1015 | 0.65 | 2.2 |
| 0.096 | 0.042 | 0.1343 | 0.60 | 1.8 |
| 0.15 | 0.060 | 0.1906 | 0.53 | 1.6 |
| 0.218 | 0.071 | 0.2519 | 0.53 | 1.5 |
| 0.3 | 0.079 | 0.3094 | 0.55 | 1.4 |
| 1.0 | 0.066 | 0.5128 | 1.04 | 1.3 |
| $C_2H_5OD + D_2O + [^2H_4]urea (x_3 = 0.09)$ | | | | |
| 0.01 | 0.006 | 0.0131 | 1.28 | 5.9 |
| 0.045 | 0.022 | 0.0559 | 0.88 | 3.4 |
| 0.069 | 0.027 | 0.0829 | 0.68 | 2.5 |
| 0.096 | 0.036 | 0.1103 | 0.65 | 2.1 |
| 0.15 | 0.054 | 0.1609 | 0.51 | 1.7 |
| 0.218 | 0.066 | 0.2149 | 0.49 | 1.5 |
| 0.3 | 0.076 | 0.2700 | 0.50 | 1.4 |

Table 3 Experimental results used for the determination of A_{22} parameters in the $C_2H_5OD + D_2O$ system at 40 °C

| $X_{C_2H_5OD}$ | $(1/T_1)_{inter}/s^{-1}$ | $c/10^{23} \text{ cm}^{-3}$ | $D/10^{-9} \text{ m}^2 \text{ s}^{-1}$ | $A/10^{-39} \text{ m}^5 \text{ s}^{-2}$ |
|----------------------------------------------|--------------------------|-----------------------------|----------------------------------------|-----------------------------------------|
| $C_2H_5OD + D_2O$ | | | | |
| 0.01 | 0.005 | 0.0162 | 1.52 | 4.8 |
| 0.045 | 0.020 | 0.0681 | 1.20 | 3.5 |
| 0.069 | 0.023 | 0.1009 | 1.09 | 2.5 |
| 0.096 | 0.030 | 0.1330 | 0.99 | 2.2 |
| 0.15 | 0.038 | 0.1887 | 0.90 | 1.8 |
| 0.218 | 0.046 | 0.2487 | 0.86 | 1.6 |
| 0.3 | 0.053 | 0.3050 | 0.87 | 1.5 |
| 1.0 | 0.047 | 0.5052 | 1.38 | 1.3 |
| $C_2H_5OD + D_2O + [^2H_4]urea (x_3 = 0.09)$ | | | | |
| 0.01 | 0.004 | 0.0130 | 1.92 | 5.9 |
| 0.045 | 0.016 | 0.0555 | 1.27 | 3.7 |
| 0.069 | 0.020 | 0.0823 | 1.06 | 2.6 |
| 0.096 | 0.028 | 0.1094 | 0.92 | 2.4 |
| 0.15 | 0.034 | 0.1593 | 0.84 | 1.8 |
| 0.218 | 0.044 | 0.2125 | 0.79 | 1.6 |
| 0.3 | 0.049 | 0.2650 | 0.80 | 1.5 |

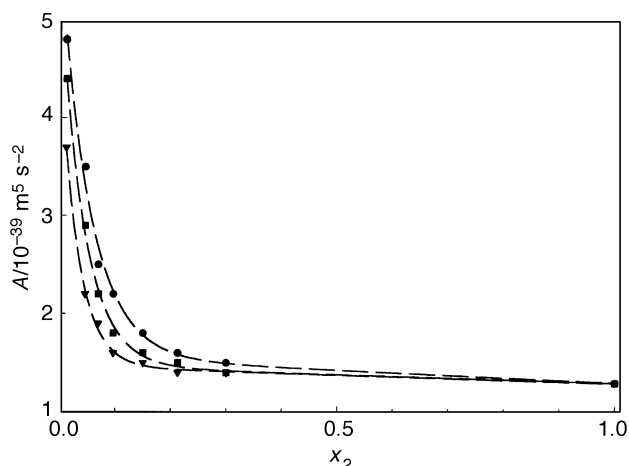
and without urea) at 10, 25 and 40 °C, are also given. We have limited our investigation in the relevant composition range, $0 < x_2 < 0.3$.

We discuss first the behaviour of the water–ethanol mixtures. In Fig. 3 we plot the values of A_{22} at 10, 25 and 40 °C in this system. The A_{22} parameter increases with decreasing x_2 , suggesting a tendency towards self-association for ethan[2H]ol. We can also notice that the A_{22} parameter increases with increasing temperature. This result is in agreement with the theoretical considerations¹⁴ and with suggestions of other authors.^{15–17} In a recent molecular dynamics study¹⁸ the temperature dependence of hydrophobic association has been investigated, showing that, in a certain temperature range, rising temperature favours association. Some of the first experimental evidence for an increase in the hydrophobic interactions with increasing temperature was reported by Matteoli and Lepori,¹⁹ our results offer further direct support. The relative increase of A_{22} with increasing temperature is stronger at lower temperatures. We can recognize this from Tables 1–3, where at $x_2 = 0.01$ we find $A_{22}(25^\circ\text{C})/A_{22}(10^\circ\text{C}) = 1.19$ whereas $A_{22}(40^\circ\text{C})/A_{22}(25^\circ\text{C}) = 1.09$. This behaviour is expected from the results of Kirkwood–Buff integrals for the same system¹⁹ supporting the suggestion of a maximum for the hydrophobic interactions at ca. 85 °C.²⁰

Compared with our results for the propanol–water system,⁷ the A_{22} parameters in Fig. 3 have comparatively high absolute values. In this context it is worthwhile noting that Nishi *et al.*²¹ recently found that ethanol in water has a surprisingly high self-association property. Studying the water–ethanol mixtures behaviour at very low ethanol concentrations ($x_2 < 0.03$) via IR spectroscopy, MS analyses of the clusters and XRD measurements, they claim that ‘the solute–solute association is highly preferable even at highly diluted concentrations’. These authors explain their results by a hydrophobic core structure composed of coherent ethyl groups with a strong hydrogen-bonding cage of water.

We now consider the water–ethanol system behaviour with the addition of urea. In Fig. 4 the behaviour at the three temperatures is reported. At all the investigated temperatures the addition of urea causes the A_{22} parameter to increase, indicating that urea promotes the ethanol molecules association.

The reason for such an increase can be ascribed to the above mentioned structure-breaking properties of urea in water. Its addition can be considered as being analogous to an increase in temperature, with respect to the water structure, at least at low alcohol concentrations. With regard to the temperature dependence of the ternary mixture, we find the same

**Fig. 3** A_{22} parameter with respect to the association of ethan[2H]ol in aqueous mixtures at 10 (▼), 25 (■) and 40 °C (●) as a function of the mole fraction of the alcohol (x_2)

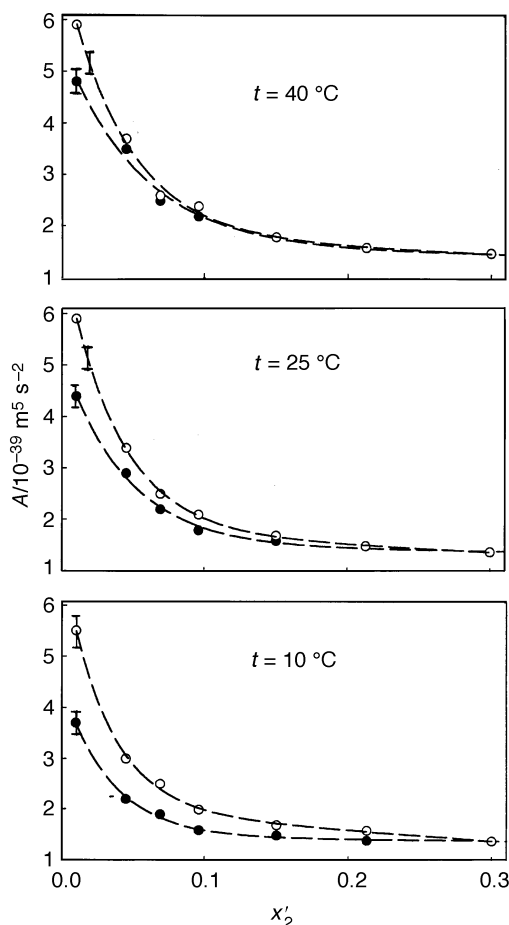


Fig. 4 A_{22} parameter with respect to the association of ethan[^2H]ol in D_2O + ethan[^2H]ol (●) and in D_2O + ethan[^2H]ol + [$^2\text{H}_4$]urea (○) mixtures at different temperatures as a function of $x'_2 = x_2/(x_1 + x_2)$

qualitative behaviour as in the pure binary mixtures, namely, a stronger relative increase at low temperatures (e.g., 48% at our reference composition at 10 °C and 23% at 40 °C).

Thus, in our opinion the addition of urea modifies the water structure in the same way as a temperature increase does and

this stronger tendency for ethanol association is also caused by an increased entropy gain.¹⁴

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