

# Evidence for Adduct Formation in the Solubilisation of Hydrophobic Compounds by Aqueous Solutions of Urea†

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The dependence on urea (U) concentration of the solubility of benzene (B) and of the neutral iron(II) complex *cis*-[Fe(*o*-phenanthroline)<sub>2</sub>(CN)<sub>2</sub>] (I) in aqueous solutions at 25 °C has been studied by u.v.-visible spectrophotometry. The increase in solubility of (I) over the range 0–4 mol dm<sup>-3</sup> urea corresponds to a single equilibrium constant  $\beta_2 = [\text{IU}_2]/[\text{I}][\text{U}]^2 = 0.7 \text{ dm}^6 \text{ mol}^{-2}$ , while the increase in solubility of B over the range 0–6 mol dm<sup>-3</sup> urea can be explained by the existence of two overlapping equilibria with constants  $K_1 = [\text{BU}]/[\text{B}][\text{U}] = 0.03 \text{ dm}^3 \text{ mol}^{-1}$  and  $K_2 = [\text{BU}_2]/[\text{BU}][\text{U}] = 0.3 \text{ dm}^3 \text{ mol}^{-1}$ . Evaluation of the published data of Wetlaufer *et al.* (*J. Am. Chem. Soc.*, 1964, **86**, 508) shows that the urea-dependent solubility of butane in water at 25 °C also corresponds to the existence of two analogous equilibria. The observation of equilibria with simple stoichiometries and with solute-dependent equilibrium constants indicates that urea exerts its solubilising effect directly through the formation of specific adducts with the solute and not indirectly by disrupting the structure of the solvent.

The ability of urea (and of the isoelectronic guanidinium cation GH<sup>+</sup>) in aqueous solution to denature proteins and to increase the solubility of hydrophobic compounds, including simple aliphatic and aromatic hydrocarbons, is well known;<sup>1</sup> in addition, we have shown that urea can significantly enhance the rate of bubble-particle attachment in the industrially important process of mineral separation by froth flotation.<sup>2</sup> This property of urea is generally ascribed to its ability to break up the ordered structure of water, *i.e.* to an effect on the properties of the bulk solvent,<sup>1</sup> but a search of the literature has failed to reveal any direct attempt to prove or disprove the alternative possibility of adduct formation by determining the stoichiometry of urea-hydrocarbon (U-HC) interactions. Most workers have simply compared solubilities in water and at one urea concentration [see, for example, ref. (3)]; we have found only two cases where a sufficiently large number of different concentrations of urea were studied to offer any prospect of testing for simple equilibria and stoichiometries, *viz.* butane<sup>3</sup> and benzene<sup>4,5</sup> (see below).

We have recently been prompted to reassess the possible formation of HC—H<sub>2</sub>O—U adducts of definite stoichiometry by (1) the common occurrence of cyclic oligomers ( $n = 5$  and 6) of H<sub>2</sub>O in samples of biological origin,<sup>6,7</sup> which may also explain the unusual stoichiometry of  $n = 12\text{H}_2\text{O}$  found for dimerisation of the water-soluble dye cobalt(II) phthalocyaninetetrasulphonate,<sup>8</sup> (2) the unusual ability of urea and GH<sup>+</sup> to form adducts (*e.g.* with H<sub>2</sub>O<sub>2</sub> and HO<sub>2</sub><sup>-</sup>, respectively)<sup>9,10</sup> through the formation of chelating hydrogen bonds, and (3) our interest in studying the effects of hydrogen bonding to coordinated cyanide in a Co corrinoid (B<sub>12</sub>) derivative,<sup>11</sup> which has led to further studies with the iron(II) complex [Fe(*o*-phenanthroline)<sub>2</sub>(CN)<sub>2</sub>] (I).

The compound I is an intensely coloured neutral complex, first prepared in 1934,<sup>12</sup> the absorption spectrum of which has been the subject of considerable interest.<sup>13–16</sup> The

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wavelength of the main absorption band in the visible region is highly solvent-dependent, moving from *ca.* 625 nm in acetone to 512 nm in water;<sup>15</sup> since the spectrum of the  $[\text{Fe}(\text{o-phenanthroline})_3]^{2+}$  ion is not solvent dependent, this can be ascribed to an interaction of the solvent with the coordinated cyanides<sup>13,16</sup> [*cf.* also ref. (11)]. It is readily soluble in organic solvents such as MeOH and  $\text{CHCl}_3$  but insoluble in benzene and ether, and rather insoluble in water. We have confirmed the assumed *cis*-conformation by X-ray diffraction.<sup>17</sup> The complex therefore possesses a hydrophobic end ( $2 \times \text{phen}$ , equivalent to six aromatic rings), which suppresses the solubility in water, and a hydrophilic end ( $2 \times \text{CN}^-$ ), which confers its finite solubility in water of *ca.*  $2.5 \times 10^{-5} \text{ mol dm}^{-3}$  (see Results), *i.e.* 0.1 % of that of benzene ( $2.3 \times 10^{-2} \text{ mol dm}^{-3}$ ).<sup>18,19</sup> It offers the possibility of studying HC–U interactions (through changes in the solubility of **I**) and simultaneously of monitoring changes in the solvent structure and the pattern of hydrogen bonding (through changes in the u.v.–visible spectrum); since it is a non-volatile solid, it is also easier to handle than benzene or the alkanes.

We report here (1) an experimental determination of the urea-dependence of the solubility of both **I** and benzene in aqueous solution at 25 °C by u.v.–visible spectrophotometry (we also used g.c. to check previous work<sup>4,5</sup> on the solubility of benzene in aqueous urea solutions), and (2) an analysis of our experimental results and of analogous published data on butane<sup>3</sup> (which all correspond to simple equilibria involving the interaction of each molecule of solute with one or two molecules of urea).

## Experimental

The complex **I** was prepared and purified (from concentrated  $\text{H}_2\text{SO}_4$ ) by the method of Schilt<sup>14</sup> to give a fine, dark-red powder, which analysed as the dihydrate. Benzene and urea were AR reagents from B.D.H. All solutions were prepared and studied at 25 °C.

Solutions of **I** were prepared by adding excess solid to 10 cm<sup>3</sup> aqueous urea solutions in a stoppered flask, which was shaken/stirred at intervals over 1 h (no further increase in absorbance observed in several experiments over a longer time span) and the excess solid filtered off to give a clear orange-coloured solution.

Solutions of benzene were prepared by placing 0.5 cm<sup>3</sup> of benzene on top of 10 cm<sup>3</sup> aqueous solution in a stoppered flask and left unshaken in the dark for over 60 h. For analysis, samples of the aqueous layer were carefully withdrawn using a Hamilton microsyringe, diluted with water to 1/10 concentration, and examined by u.v.–visible spectrophotometry in a 5 mm pathlength cell.

Spectra were recorded, and optical densities measured, with either a Phillips PU 8740 or a Pye-Unicam SP 8100 spectrophotometer. Gas chromatography was carried out with 10<sup>−3</sup> cm<sup>3</sup> samples of aqueous solutions of benzene using Porapak Q with the oven temperature at 190 °C, flame ionisation detector at 250 °C, and N<sub>2</sub> as the carrier gas at a rate of 40 cm<sup>3</sup> min<sup>−1</sup>.

## Results

### **I** and Urea

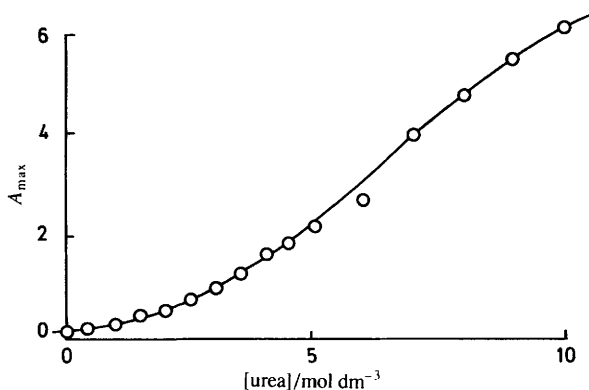
Solutions were prepared as described (Experimental). Initial experiments established (i) that the wavelength of the main absorption band in the visible region was shifted slightly but regularly to longer wavelength with increasing concentration of urea (from 513 nm in pure water to 519 nm in 10 mol dm<sup>−3</sup> urea), (ii) that Beer's Law was obeyed (up to  $7 \times 10^{-5} \text{ mol dm}^{-3}$  **I**) in 1, 4 and 10 mol dm<sup>−3</sup> urea solutions, *i.e.* we are probably dealing only with monomers of **I** under all conditions studied, and (iii) that the molar extinction coefficient at the wavelength of the absorption maximum remained unchanged (within 5 %) at  $6 \times 10^3$  from 0–10 mol dm<sup>−3</sup> urea.

Experimental results for the variation of the solubility of **I** (given as the observed  $A_{\text{max}}$ ) with varying urea concentration are listed in table 1 and plotted in fig. 1. The

**Table 1.** Solubility of **I** in aqueous urea solutions at 25 °C<sup>a</sup>

[urea]/mol dm <sup>-3</sup>	<i>A</i> <sub>max</sub> <sup>b</sup>
0	0.16
0.5	0.21
1.0	0.25
1.5	0.49
2.0	0.57
2.5	0.79
3.0	1.06
3.5	1.31
4.0	1.78
4.5	1.93
5.0	2.29
6.0	2.76
7.0	4.05
8.0	4.91
9.0	5.60
10.0	6.22

<sup>a</sup> For details see text. <sup>b</sup> Solutions with *A*<sub>max</sub> > 2 were analysed after dilution; the values given refer to the derived *A*<sub>max</sub> of the undiluted solutions.

**Fig. 1.** Variation of the solubility of **I** (given as *A*<sub>max</sub>) with urea concentration. Data from table 1.

non-zero origin shows that **I** has a small but finite solubility in water, calculated from *A*<sub>molar</sub> to be  $2.5 \times 10^{-5}$ . The sigmoid shape of the curve with a point of inflection at *ca.* 7 mol dm<sup>-3</sup> urea indicates the existence of (at least) two equilibria. Fig. 2 shows the plot of solubility against the square of the urea concentration up to 4 mol dm<sup>-3</sup> urea; the linearity of the plot indicates that urea solubilises **I** according to equilibrium (1) (below) involving two molecules of urea per molecule of **I**. The total observed solubility in this region is therefore the sum of the concentrations of the two species **I** and **IU**<sub>2</sub>. Since **[I]** = **[IU**<sub>2</sub>] in 1.2 mol dm<sup>-3</sup> urea, the value of the equilibrium constant  $\beta_2 = [\text{IU}_2]/[\text{I}][\text{U}]^2 = 1/(1.2)^2 = 0.7 \text{ dm}^6 \text{ mol}^{-2}$ .



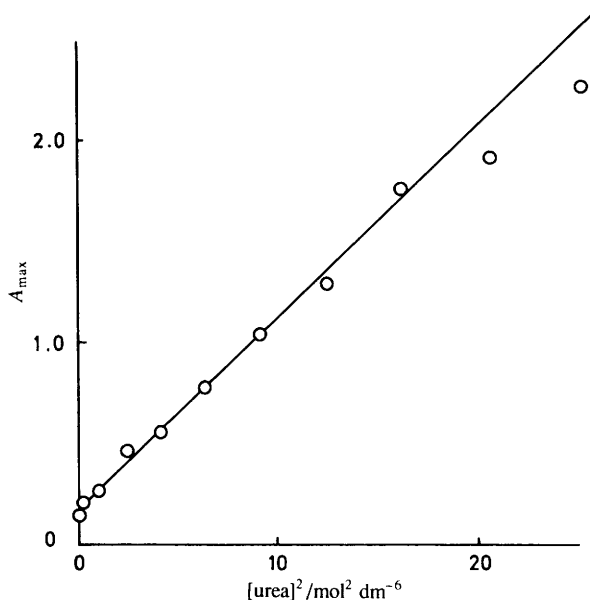


Fig. 2. Dependence of the solubility of I (given as  $A_{\text{max}}$ ) on the square of the urea concentration. Data from table 1.

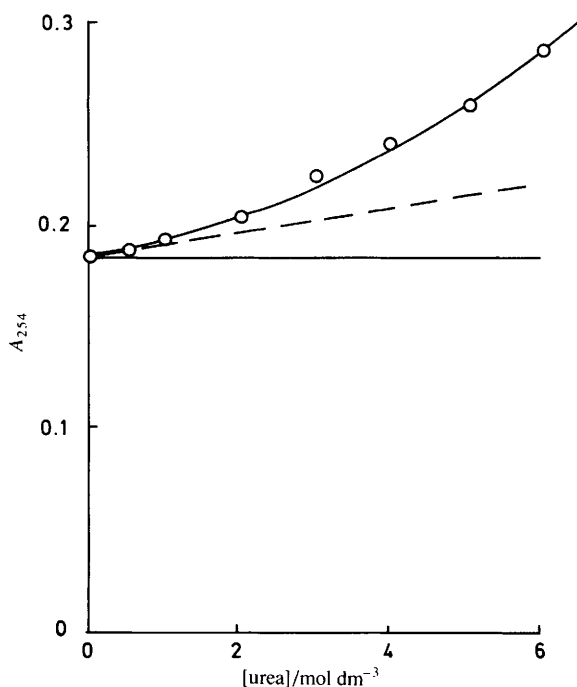
The most likely explanation for the reverse curvature above  $7 \text{ mol dm}^{-3}$  urea is that  $\text{H}_2\text{O}$  is required for the dissolution of I and/or for the further association of I with urea, and that the use of such high concentrations of urea causes a significant decrease in the concentration of  $\text{H}_2\text{O}$  (to *ca.*  $1/3$  in  $10 \text{ mol dm}^{-3}$  urea), irrespective of any additional changes in the structure of the solvent or the activity of  $\text{H}_2\text{O}$ . Similar deviations may be expected with other solutes above  $5 \text{ mol dm}^{-3}$  urea (see benzene below).

### Benzene and Urea

Initial experiments were aimed at checking the results of Bittrich *et al.*<sup>4,5</sup> who used g.c. to determine the solubility of benzene in aqueous solutions of urea ( $0$ – $11 \text{ mol dm}^{-3}$ ) at  $25$ ,  $40$ , and  $60^\circ\text{C}$ . Their tabulated data for  $25^\circ\text{C}$  indicate a surprising break at *ca.*  $3 \text{ mol dm}^{-3}$  urea from constant solubility to rapidly increasing solubility, but the authors offer no explanation or even comment. Our experiments using g.c. (see Experimental) with solutions up to  $10 \text{ mol dm}^{-3}$  urea gave results approximating to the curve shown below (fig. 3) and did not display the reported discontinuity; but the errors were too large for the necessary analysis and g.c. was therefore abandoned in favour of u.v.–visible spectrophotometry for determining the concentration of benzene in solution.

Solutions of benzene in aqueous urea were prepared as described (Experimental) and samples diluted with water to  $1/10$  concentration before recording the absorbance of the  $254 \text{ nm}$  band in a  $5 \text{ mm}$  pathlength cell. It was shown that both the wavelength and the molar extinction coefficient of the  $254 \text{ nm}$  band were unaffected by the presence of  $1 \text{ mol dm}^{-3}$  urea (maximum concentration present at the stage of analysis), Beer's Law was assumed, and the molar extinction coefficient of  $A_{254} = 164$  determined from the observed value of  $A_{254}$  of a saturated solution of benzene in pure water and the published solubility of benzene in water at  $25^\circ\text{C}$  ( $2.3 \times 10^{-2} \text{ mol dm}^{-3}$ ).<sup>18,19</sup>

Experimental results for the variation of solubility of benzene, given as the observed  $A_{254}$ , are presented in table 2 and plotted against the urea concentration in fig. 3. A



**Fig. 3.** Variation of the solubility of benzene (given as  $A_{254}$ ) with urea concentration. Data from table 2.

**Table 2.** Solubility of benzene in aqueous urea solutions at 25 °C<sup>a</sup>

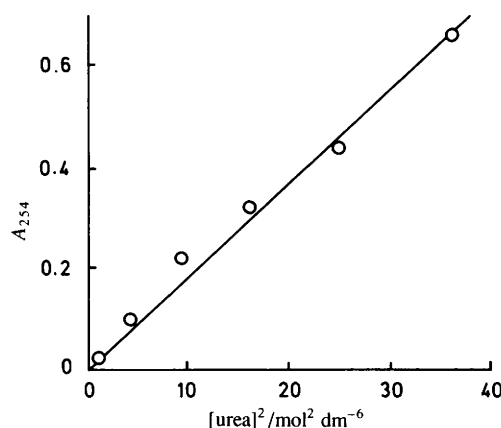
[urea]/mol dm <sup>-3</sup>	$A_{254}$ <sup>b</sup>	contribution to $A_{254}$ from BU <sub>2</sub> species
0	0.187	—
0.5	0.192	—
1	0.195	0.002
2	0.207	0.008
3	0.227	0.022
4	0.243	0.032
5	0.261	0.044
6	0.290	0.067

<sup>a</sup> For details see text. <sup>b</sup> Values of  $A_{254}$  represent the average of 2 or 3 independent measurements with a maximum scatter of  $\pm 0.003$ .

decrease in curvature was noted above 6 mol dm<sup>-3</sup> urea (*cf.* results with **I**) and results at higher concentrations were therefore discounted. The tangent at  $[U] = 0$  has a finite slope (contrast fig. 1), as shown by the dashed line. This suggests that urea solubilises benzene according to the two equilibria.



The relative contributions to the total observed solubility of benzene from the three species B, BU and BU<sub>2</sub> correspond to the heights from the  $x$  axis to the solid line, from



**Fig. 4.** Dependence of the concentration of the presumed  $\text{BU}_2$  adduct of benzene (given as  $A_{254}$ ) on the square of the urea concentration. Data from table 2.

**Table 3.** Solubility of butane in aqueous urea solutions at 25 °C<sup>a</sup>

[urea]/mol dm <sup>-3</sup> <sup>b</sup>	solubility of butane 10 <sup>3</sup> [C <sub>4</sub> H <sub>10</sub> ]/mol dm <sup>-3</sup>	contribution from $\text{BU}_2$ species 10 <sup>3</sup> [BU <sub>2</sub> ]/mol dm <sup>-3</sup>
0	1.17	—
2	1.23	—
3	1.25	0.05
4	1.30	0.09
6	1.38	0.14
7	1.45	0.20
8	1.53	0.26
10	1.64	0.34

<sup>a</sup> Data taken from fig. 4 of ref 3. <sup>b</sup> Molarities of the urea solutions assumed to be integral. For further details see text.

the solid to the dashed line, and from the dashed to the experimental line, respectively. The contributions to  $A_{254}$  from  $\text{BU}_2$  are listed in table 2 and plotted against the square of the urea concentration in fig. 4; the reasonable linearity of the plot, in spite of small differences and relatively large errors, supports the correctness of eqn (3) and hence of eqn (2). The data of table 2 and fig. 3 lead to values of  $K_1 = [\text{BU}]/[\text{B}][\text{U}] = 1/30 = 0.03 \text{ dm}^3 \text{ mol}^{-1}$ , and  $K_2 = [\text{BU}_2]/[\text{BU}][\text{U}] = 1/3.5 = 0.3 \text{ dm}^3 \text{ mol}^{-1}$ , hence  $\beta_2 = K_1 K_2 = \text{ca. } 0.01 \text{ dm}^6 \text{ mol}^{-2}$ .

### Butane and Urea

Wetlaufer *et al.*<sup>3</sup> have studied the variation of the solubility of butane with urea concentration at 25 °C and shown the results graphically in fig. 4 of their paper; though not stated explicitly in the text, it is clear by comparing fig. 4 with table 1 that the data refer to 25 °C. Our readings from their figure are listed in table 3 and plotted against urea concentration in fig. 5. As in the case of benzene, the tangent at 0 mol dm<sup>-3</sup> urea clearly has a finite slope and we interpret the plot to indicate the existence of the three analogous species (B, BU,  $\text{BU}_2$ ). If the tangent is drawn from  $1.17 \times 10^{-3}$  to  $1.30 \times 10^{-3} \text{ mol dm}^{-3}$  butane in 0 and 10 mol dm<sup>-3</sup> urea, respectively, the plot of the contribution of the  $\text{BU}_2$

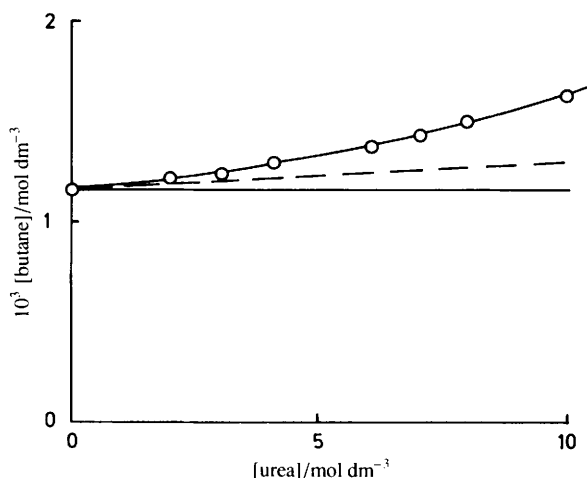


Fig. 5. Variation of the solubility of butane with urea concentration. Data from table 3.

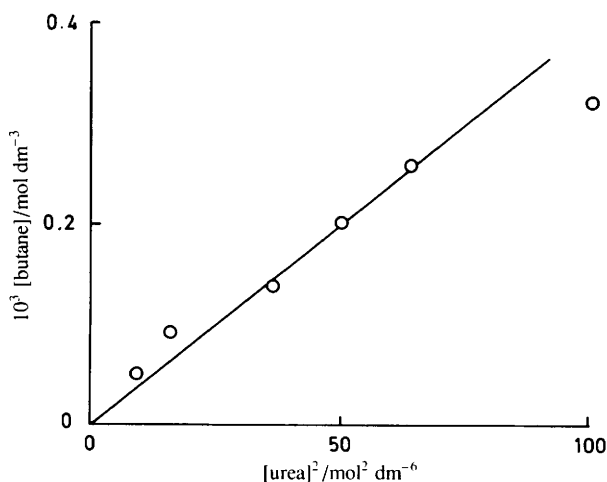


Fig. 6. Dependence of the concentration of the presumed  $\text{BU}_2$  adduct of butane on the square of the urea concentration. Data from table 3.

species (see table 3) against the square of the urea concentration (see fig. 6) is reasonably linear up to at least  $8 \text{ mol dm}^{-3}$  urea, *i.e.* supports the existence of equilibria analogous to (2) and (3). The data can be used to give rather approximate values of the equilibrium constants  $K_1 = ca. 1 \times 10^{-2} \text{ dm}^3 \text{ mol}^{-1}$  and  $K_2 = ca. 0.3 \text{ dm}^3 \text{ mol}^{-1}$ .

## Discussion

The data of tables 1 and 2 and fig. 1–4 show that the urea-induced increase in solubility can be explained by the existence of simple equilibria involving the interaction of **I** with two molecules of urea and of benzene with both one and two ureas. Table 3 and fig. 5 and 6 show that, within experimental error, the published data on butane also agree with the existence of two equilibria analogous to those observed for benzene. The same paper provided data on the solubility of butane in aqueous solutions of  $\text{GH}^+$ ; the plot in fig. 4 of ref. (3) shows a pronounced upward curve which indicates interaction with

**Table 4.** Summary of equilibrium constants for adduct formation

	$K_1/\text{dm}^3 \text{ mol}^{-1}$	$K_2/\text{dm}^3 \text{ mol}^{-1}$	$\beta_2 (= K_1, K_2)/\text{dm}^6 \text{ mol}^{-2}$
I-urea	—	—	0.7
benzene-urea	0.03	0.3	0.01
butane-urea <i>ca.</i>	0.01	<i>ca.</i> 0.3	$10^{-3}$ – $10^{-2}$

more than one molecule of  $\text{GH}^+$  but, since the ionic strength was not kept constant, the results cannot be properly analysed. We have also found that  $\text{GH}^+$  increases the solubility of **I** and the results correspond to interaction of **I** with two  $\text{GH}^+$  ions (to be published after studying benzene with  $\text{GH}^+$ ). All presently available evidence serves to confirm the previously stressed<sup>3</sup> similarity in behaviour of both alkanes and aromatic compounds towards both urea and  $\text{GH}^+$  and, in addition, demonstrates that **I** can serve as a convenient hydrophobic ‘model’ for studying these interactions.

Values for the equilibrium constants involving butane, benzene and **I** are summarised in table 4. The fact that the values are solute-dependent means that the observed increase in solubility cannot be caused indirectly by, for example, the formation of urea dimers which disrupt the structure of the solvent, but must be caused directly by the formation of adducts between urea and the solute. As far as we are aware, these represent the first established examples of  $\text{HC}$ -urea (or  $\text{HC}$ - $\text{GH}^+$ ) adduct formation in solution. It seems reasonable to presume similar adduct formation in all other cases where urea or  $\text{GH}^+$  enhances the solubility of such hydrophobic compounds and to modify our views on the role of urea accordingly. Possible structures for such  $\text{HC}$ -urea adducts are best discussed after obtaining evidence for the number of  $\text{H}_2\text{O}$  molecules (if any) involved in the adduct.

Table 4 reveals the rather surprising fact that  $K_1 \leq K_2$  in all three cases; if the two ureas were bound independently (*e.g.* on opposite sides of the benzene ring), one would expect  $K_1 = 4K_2$  on statistical grounds.<sup>20</sup> The observed order may therefore indicate some cooperativity between the binding of the two molecules of U or  $\text{GH}^+$ , perhaps mediated by the presence of one or more  $\text{H}_2\text{O}$  molecules in the adduct. A study of the factors which influence the ratio  $K_1/K_2$  could therefore provide additional clues about the structure of the adducts.

Finally, we note that the reason why the complex **I** has provided the first experimental evidence which so emphatically suggests an ‘adduct-making’ rather than a ‘structure-breaking’ role for urea (see fig. 1) is related to the magnitude of the equilibrium constants; the unusually high value of  $\beta_2$  enables a considerable part of the curve for adduct formation to be observed before the onset (at *ca.*  $5 \text{ mol dm}^{-3}$  urea) of other complicating factors, while the high ratio of  $K_2/K_1$  is associated with a characteristic upward curve from a horizontal tangent, which could hardly be confused with a more general effect of urea on the structure of the solvent.

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## References

- 1 *Water: A Comprehensive Treatise*, ed. F. Franks (Plenum Press, London, 1975) vol. 2 and 4.
- 2 G. Brill and J. M. Pratt, *Int. J. Miner. Process* 1979, **6**, 173.
- 3 D. B. Wetlaufer, S. K. Malik, L. Stoller and R. L. Coffin, *J. Am. Chem. Soc.*, 1964, **86**, 508.
- 4 H. Bittrich, H. Gedan and G. Feix, *Z. Phys. Chem.*, 1979, **260**, 1009.
- 5 H. Bittrich, A. Fahl and F. Hartwig, *Z. Phys. Chem.*, 1983, **264**, 891.
- 6 W. Saenger, *Nature (London)*, 1979, **279**, 343.



- 7 S. Neidle, H. M. Berman and H. S. Shieh, *Nature (London)*, 1980, **288**, 129.
- 8 E. W. Abel, J. M. Pratt and R. Whelan, *J. Chem. Soc., Dalton Trans.*, 1976, 509.
- 9 C. J. Fritchie and R. K. McMullan, *Acta Crystallogr. Sect. B*, 1981 **37**, 1086.
- 10 D. A. Baldwin, H. M. Marques and J. M. Pratt, *FEBS Lett.*, 1985, **183**, 309.
- 11 A. J. Markwell, J. M. Pratt, M. S. Shaikjee and J. G. Toerien, *J. Chem. Soc., Dalton Trans.* 1987, 1349.
- 12 G. A. Barbieri, *Atti Accad. Lincei*, 1934, **20**, 273.
- 13 J. Bjerrum, A. W. Adamson and O. Bostrup, *Acta. Chem. Scand.*, 1956, **10**, 329.
- 14 A. A. Schilt, *J. Am. Chem. Soc.*, 1960, **82**, 3000.
- 15 J. Burgess, *Spectrochim. Acta, Part A* 1970, **26**, 1369.
- 16 H. E. Toma and M. S. Takasugi, *J. Solution Chem.*, 1983, **12**, 547.
- 17 V. Ramdas, unpublished results.
- 18 P. M. Gross and J. H. Saylor, *J. Am. Chem. Soc.*, 1931, **53**, 1744.
- 19 R. L. Bohon and W. F. Claussen, *J. Am. Chem. Soc.*, 1951, **73**, 1571.
- 20 R. P. Bell, *The Proton in Chemistry*, (Methuen, London, 1959), pp. 97, 98.

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