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Thermochemical investigation of possible interactions between urea and some sparingly soluble solutes in aqueous solution

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Abstract

The interaction of alkyl-*p*-hydroxybenzoates and of β-phenylalanine with urea in dilute aqueous solution which has been proposed by some workers, has been investigated by microcalorimetry. The derived enthalpies are all less than -50 J mol^{-1} and hence are too small to be considered as describing specific interactions between urea and the study solutes. Furthermore a linear relationship is shown to exist for a wide variety of compounds, including the study solutes, between solute size (surface area and molecular or van der Waals' volume) and $\Delta_{trs} G$, the Gibbs function for solute transfer from water to aqueous urea solution. Both these findings support the view that the effect of urea upon these solutes' aqueous solubility is an entropically controlled bulk solvent process rather than specific complex formation between urea and solute. The results are discussed in the light of recent models of the hydrophobic effect which propose displacement by urea of water solvation molecules in the solute's hydration shell.

Keywords: Alkylhydroxybenzoate; Gibbs function; Phenylalanine; Solute transfer; Urea

1. Introduction

Urea solutions are of considerable importance and interest both fundamentally and practically. The interactions of sparingly soluble solutes in aqueous solutions of

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urea are also of contemporary interest at both the theoretical and practical level. For instance Muller has described [1,2] a theoretical model of hydrophobic hydration which incorporates the addition of a urea-like cosolvent to the studied system. The model developed suggests that the cosolvent reduces hydrophobic hydration by a purely geometric mechanism. Thus the cosolvent results in the reduction of the average number of hydration-shell bonds involved with each solute molecule; this is a purely steric phenomenon. This result naturally requires that the thermodynamic parameters deducible from the model are likewise affected. There is, furthermore, modification of the van der Waals' interactions. Muller [2] has therefore, made "appropriate" choices of the cosolvent molar volume and of the model-required empirical parameters to arrive at agreement between calculated and experimentally derived values for the enthalpies, Gibbs functions and entropies of solute transfer from water to 7 mol dm^{-3} urea solutions. The cited references [1,2] also give a review of the literature in this area which can be consulted for further detail.

Approximately 7 mol dm^{-3} solutions of urea are also used for the denaturation of proteins and the unique interaction of urea with water, usually referred to as structure-breaking, results in the collapse of protein quaternary structure and hence in denaturation. Detailed accounts of the interaction of proteins with urea are, of course, readily available [3].

However, at concentrations of urea very much lower (typically about 0.1 mol dm^{-3} and less than 2 mol dm^{-3}) than 7 mol dm^{-3} there is experimental evidence for a variety of interactions between urea and added solutes; for example, for an increase [4] in the pK_a of weak acids, for the increase in solubility of the alkyl-*p*-hydroxybenzoates [5] (parabens), of amino acids [6] and of alkanes [7]. These last four reports suggest that the reason for the effects noted is the existence of urea–solute complexes. The complexes described between the parabens [5], the amino acids [6] and the alkanes [7] are of urea–solute and of $(urea)_2$–solute stoichiometry. These conclusions are based only upon analysis of the experimental observations of linear or curvilinear relationships between solubility variation and urea concentration. Furthermore, Kresheck and Benjamin [8] have reported a calorimetric investigation of the interaction of some amino acids with urea. The data produced were for interaction with urea at concentrations of 6 mol dm^{-3} (some experiments were also conducted in 2 mol dm^{-3} urea solution). The conclusions here at these much higher urea concentrations were that the interaction was mainly mediated through the effects of urea upon the solvent–water structure, although there was said to be evidence for some specific interaction. Sijpkes et al. [10] have studied the interaction of diketopiperazine (DKP) with urea (U) in aqueous solution through solubility measurements. They suggest the formation of a co-crystal of $DKP \cdot U_2$ at urea activities greater than 5.7 ± 0.2 mol kg^{-1}.

The derived association constant for the formation of a peptide–urea species was rather small (0.0447 ± 0.0007 kg mol^{-1}). Indeed, from aqueous urea solutions of concentrations above 6 mol dm^{-3} a co-crystal of $DKP \cdot U_2$ has been prepared and its structure resolved [11]. The proposed existence of a formal complex species upon interaction of the described solutes with dilute urea solution ought to be examinable via microcalorimetry and the experimental observation of the enthalpies associated

with such complex formation. It is the purpose of the work reported in this paper to explore the nature and existence of such complexes through thermochemical experiments. Thus the results may contribute to establishing the possible existence of such complexes, their stoichiometry and to evaluating the development of theoretical models of the hydrophobic effect and to the role of urea in such systems.

2. Experimental methods and results

2.1. Chemicals

Methyl, ethyl, propyl and pentyl-*p*-hydroxybenzoate were purchased from Sigma, Koch-Light, BDH and Apin Chemicals, respectively. *β*-Phenylalanine was purchased from BDH. All these materials have a stated purity of better than 99% and were used as received. Urea, 98%, was supplied by Aldrich. The water used throughout this study was doubly distilled and deionised.

2.2. UV absorption spectroscopy

UV measurements were performed in a diode array spectrophotometer, Hewlett-Packard 8452A, with a Peltier temperature controller ($T \pm 0.1$ K) operated according to the manufacturer's instructions.

The pK_a of the various parabens [5] in water is 8.40 and the addition of urea leads to their ionisation because of the weak base character of the urea (Fig. 1). The same degree of ionisation was observed for all the parabens species studied. UV absorption was also used to monitor the variation in urea solution properties with time and Fig. 2 shows the changes in the UV spectrum for a paraben in 0.35 mol dm^{-3} urea solution left in an open cuvette. In the light of these observations all spectra and investigations were conducted using freshly made urea solutions. Buffered urea solutions were also investigated as solvent (see below).

2.3. Microcalorimetry

The interaction (at 298 K) between the study solutes and urea was investigated using an LKB 10700 batch microcalorimeter which was adapted to also perform in the titration mode. Its operation was as prescribed in the manufacturer's manual. Calibration was by the electrical substitution method. The smallest signals recorded were in the order of 0.5–2.0 mJ and the reproducibility obtained in electrical calibration in this range was better than 2%.

Microcalorimetric titrations (typically four separate experiments for each solute, with each experiment consisting of 10 titrant additions) were performed in both aqueous and in buffered urea solution. The maximum urea concentration employed was generally 0.04 mol dm^{-3} in order to avoid significant solute ionisation as noted above. Methyl- and ethylparaben concentrations were 10.7 and 5.08 mol dm^{-3} respectively (close to their solubility limits in water). The enthalpies of interaction,

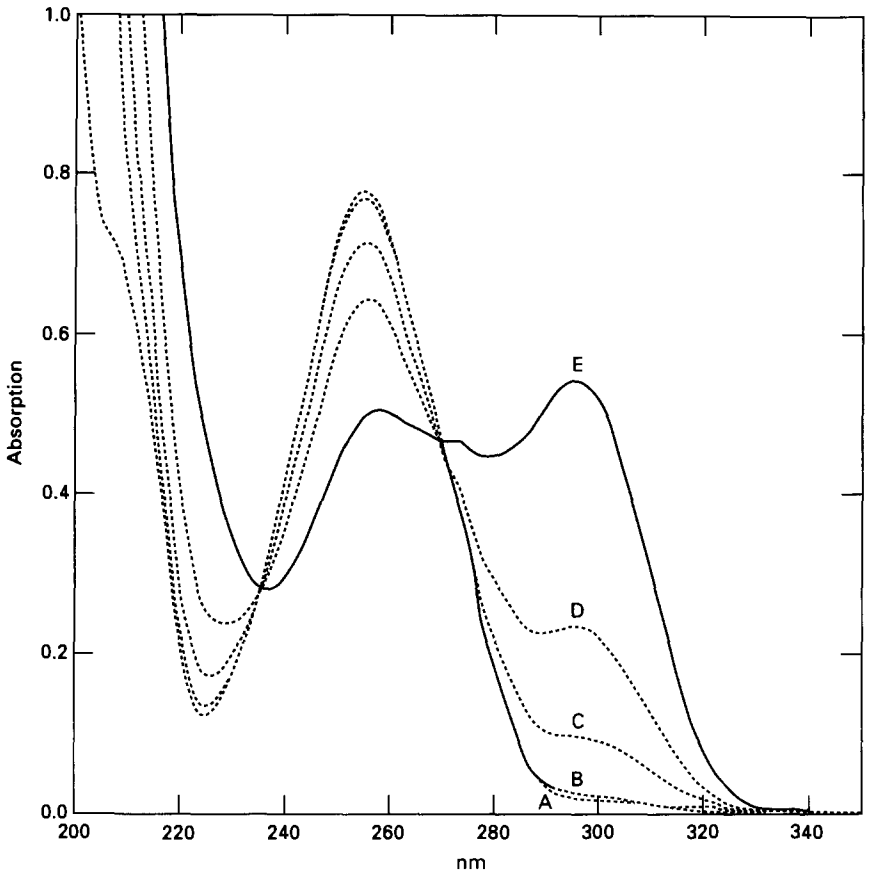

Fig. 1. UV absorption spectra of methylparaben 4×10^{-5} mol dm^{-3} as a function of increasing urea concentration. Curve A, no urea; curve B, 0.044; curve C, 0.088; curve D, 0.18; curve E, 0.35 mol dm^{-3}.

discounting (from measurement and subsequent analysis of data) the enthalpies of dilution of urea and of the parabens were, for methyl- and ethylparaben respectively, -21 ± 9 and -19 ± 10 J mol^{-1}.

The interaction of methylparaben and urea was investigated at higher urea concentrations in the presence of 0.1 mol dm^{-3} phosphate buffer at pH 6.0. Under these conditions, with urea concentrations up to 2 mol dm^{-3}, the UV spectrum of methylparaben presented no evidence of ionisation. These conditions are similar to those used by Dempsey and Molyneux [5] (they state only Sorenson phosphate buffer was used and hence imply a buffer concentration of about 0.07 mol dm^{-3}). For urea solutions up to 0.1 mol dm^{-3} and with methylparaben at a concentration of 7.36 mmol dm^{-3} the determined enthalpy for interaction was -40 ± 14 J mol^{-1}. Some experiments with 2 mol dm^{-3} urea produced results within the ranges quoted above. The interaction between urea and β-phenylalanine was also studied by titration microcalorimetry. The measurements were performed in 0.1 mol dm^{-3}

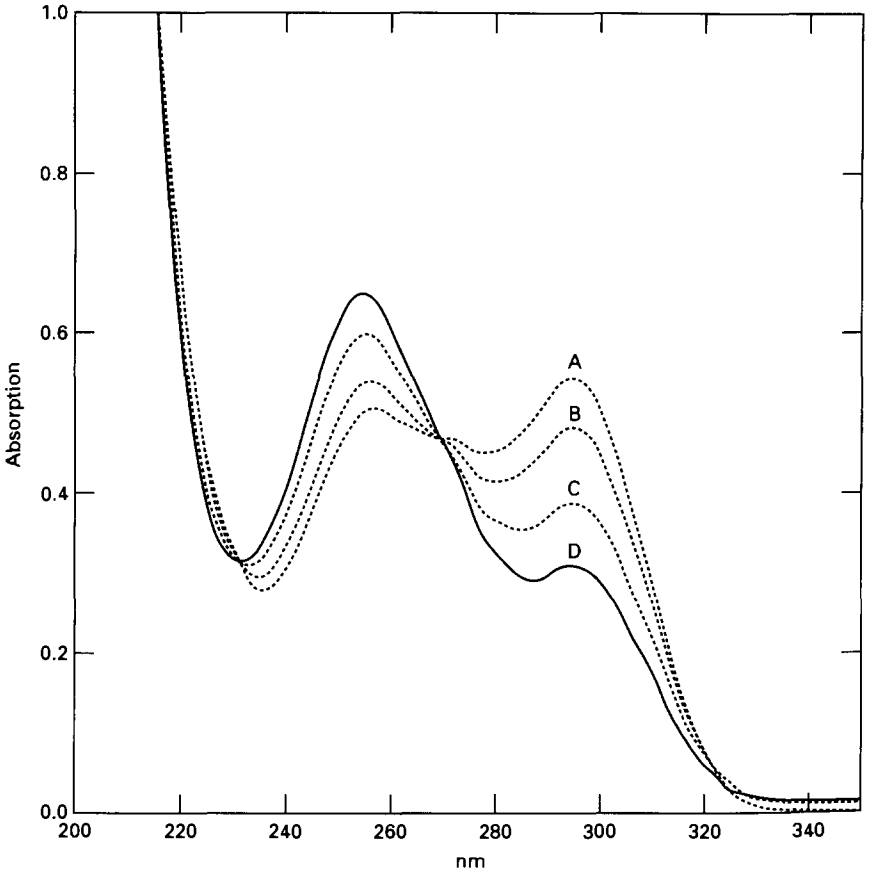

Fig. 2. Change in the UV spectra of methylparaben 4×10^{-5} in urea 0.35 mol dm^{-3} left in an open cuvette. Curve A, 30 min; curve B, 75 min; curve C, 120 min; curve D, 180 min after preparation.

phosphate buffer of pH 5.9 (the isoelectric point of this amino acid). Urea was titrated up to 0.3 mol dm^{-3} in the presence of 0.1 mol dm^{-3} β-phenylalanine. The enthalpy for this interaction was determined as -31 ± 8 J mol^{-1}.

3. Discussion

The values for the interaction enthalpies determined in this investigation are all less than -40 J mol^{-1} with an associated uncertainty which is about ± 10 J mol^{-1}. Such a small molar enthalpy is lower than any conceivable form of interaction (e.g. H-bond, van der Waals' dispersion) between urea and solute. The average thermal energy at 298 K is estimable as 3.7 kJ mol^{-1} and this means that a urea–solute complex is weaker than any normal solute–solute interaction and would easily be disrupted by thermal motion. The fact that an enthalpy can be recorded at this low

level is a reflection of the sensitivity of the microcalorimeter and of the difficulties in ensuring absolutely balanced output in the differential form of the microcalorimetric experiment. There is, from these results, no thermochemical evidence to support the existence of complex formation between urea and the parabens and between urea and the amino acids (assuming β-phenylalanine is representative of amino acid behaviour with respect to interaction with urea). However, it is the case that there exists experimental evidence for the effects of urea upon the solubilities of the study solutes. There is, therefore, the need to explore the nature of the observed effects in the absence of direct experimental evidence for complex formation. An alternative approach to the interpretation of the experimental solubility data is to consider that the increased solubilities may result from the consequence of bulk effects rather than from specific interaction as would be required in the complex formation account. Indeed Dempsey and Molyneux [5] give some general consideration to this explanation but their final conclusion from solubility data alone is that complexation accounts for the observations. Muller's theoretical model [1,2], as briefly described in the Introduction, involves the purely geometric incorporation of urea-like cosolvents into the hydration shell. Muller [2] states that urea, like cosolvents, acts "by preempting space in the solvation shell that would otherwise accommodate water molecules". This type of account for the systematic variation in properties is, of course, not new (although the model is). Variation in solution phase properties has, for some long time, revealed relationships between measured variables (e.g. solubility, partition coefficient, etc.) and geometric properties of solutes such as molar volume and solute surface area. For example, Lilley [15] has discussed in detail the thermodynamics of peptides and model systems and explores the nature of bulk effects and "structural" effects in urea solutions. Schellman [12] has recently concluded that solvent components such as urea and guanidinium chloride affect "interaction" through profound effects upon activity coefficients. Such an explanation is sufficient, within the terms of the model, to account for weak interactions. That is, in the circumstances of the investigations outlined in the Introduction, it is not necessary to invoke specific interaction: it is only required that the solvent component's activity coefficients vary significantly. The data reported here, in general, support the view that non-specific effects are sufficient to account for the experimental observations. Calculation of the surface areas and molar volumes from published [13] data for the study solutes allows exploration of the variation in the Gibbs function for transfer of solute from water to urea solution with both these parameters. The molecular areas and volumes of these solutes were estimated from their van der Waals' volumes. Although more refined methods have been proposed for these estimates in the case of the homologous series of parabens, the atomic contributions lead [9] to the same van der Waals' volume calculated using a small modification of the procedure proposed by Gaudio and Takahata [13]. In addition, this simple method provides a self consistent data set. Applying this procedure to the data on the parabens and the amino acid data of Nozaki and Tanford [14] allows exploration of the existence of any bulk effects that may contribute to an interpretation of the observed solubility variations as a result of change in the urea concentration. The resulting plots are

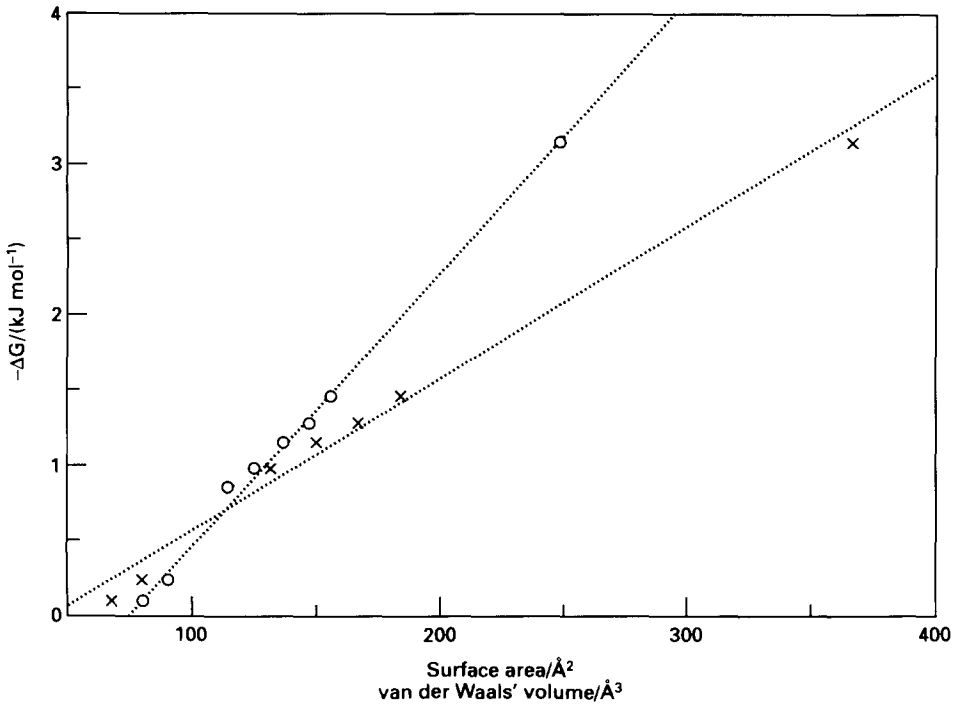

Fig. 3. Change in Gibbs function for solute transfer ($\Delta_{trs}G$) from water to 2 mol dm^{-3} urea solution vs. solute surface area (○) and solute van der Waals' volume (×) for butane, benzene, *p*-hydroxybenzoate, methyl-, ethyl-, propyl- and butyl-*p*-hydroxybenzoate and Fe(*o*-phen)$_2$CN$_2$. Data from Refs. [5] and [7]. Best fit parameters: surface area, intercept = −1.29; slope = 0.018 and r = 0.998; van der Waals' volume, intercept = 0.40; slope = 0.010 and r = 0.992.

displayed in Fig. 3. It is immediately apparent from this figure that the change in solubilities can be related, in a linear fashion, to either of these geometric quantities. The linear relationship is revealed to cover solutes as different as butane [7], benzene [7], the monoanion of *p*-hydroxybenzoic acid [5], the parabens [5] and the complex species Fe(*o*-phen)$_2$CN$_2$ [7]. The data for these radically different solutes all lie on the same line. The amino acid data [14], however, lie on another line of identical slope but with a different intercept: see Fig. 4, which also shows a similar plot for guanidinium hydrochloride–amino acid data [14]. Such behaviour indicates that any increase in solubility induced by urea (and by guanidinium hydrochloride) is related to the size of the cavity within the water structure needed to accommodate the solute. The different line found for the amino acids may be attributed to their larger hydrophilicity due mainly to their electrostatic charges and small sizes. Because the enthalpy contribution to the transfer of solute from water to aqueous urea solution is, from the data reported here, essentially zero, then the experimentally observed variation in the Gibbs function must be attributed to entropic effects alone. Note that the data used to construct Fig. 3 were drawn from the published data [5–7,14] and hence relate to urea concentrations up to 2 mol dm^{-3} and not

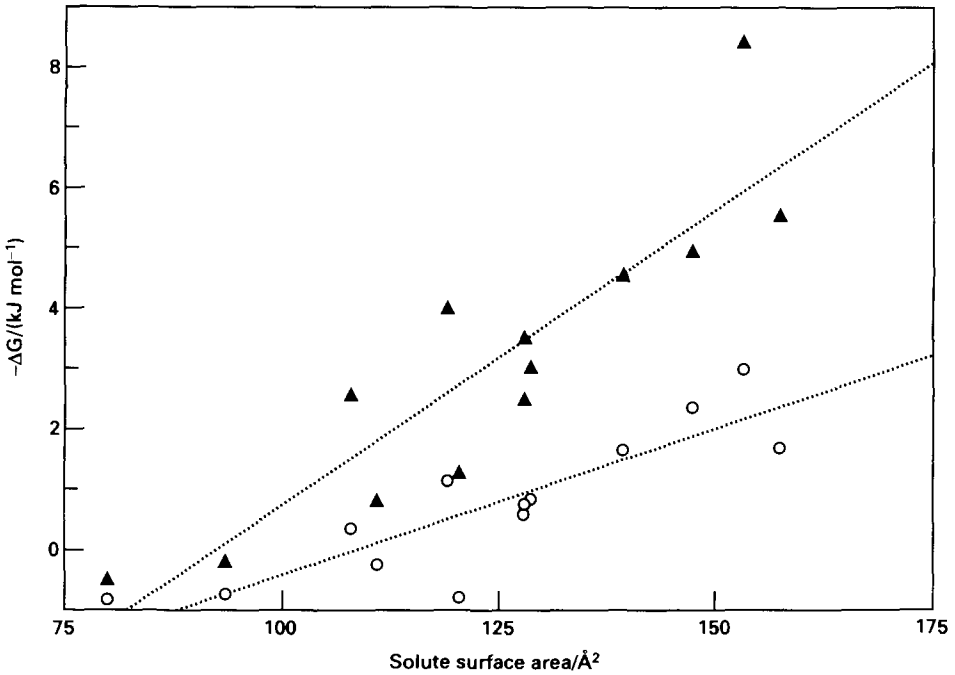

Fig. 4. $\Delta_{trs}G$ for the transfer of amino acids from water to 2 mol dm^{-3} urea (▲) and 2 mol dm^{-3} guanidinium hydrochloride (○) vs. solute surface area. Best fit parameters; urea, intercept = −5.0; slope = 0.047 and $r = 0.873$; GuHCl, intercept = −9.0; slope = −9.0 and $r = 0.893$. Data from Ref. [1].

merely to the concentrations of urea reported in the Experimental section as being used in this work. Thus, that at low urea concentration there is no thermochemical evidence for complex formation is reinforced by the transformation of the literature data (where [urea] ≤ 2 mol dm^{-3}) into a simple, linear dependence upon solute molecular dimensions.

Entropic effects in aqueous solution are the origins of the hydrophobic effect. The picture which emerges from these effects on solubilities in aqueous urea solutions is that urea molecules preferentially surround the solute reducing the unfavourable water–apolar solute contacts. Because urea has a strong hydrogen bonding capacity its insertion into water structure does not greatly perturb this structure, leading to an energetic balance between disruption and formation of hydrogen bonds; this is reflected in the essentially zero enthalpies reported in this work.

The existence of a linear relationship between the Gibbs function for transfer of solute from water to aqueous urea solution argues for a purely bulk solvent effect to account for the experimental observations of solubility variation with urea concentration. Taken together with the complete absence of thermochemical evidence for complex formation we conclude that these solutes do not form complexes with urea and that the variation in their solution phase properties is solely a result of solvation shell changes consequent upon addition of urea. As noted above, such

findings are also consistent with other investigations of the thermodynamics of solutions containing urea. Thus for example, Lilley [15] argues for bulk effects in analysing the thermodynamic consequences for peptides of the presence of urea in solution.

The conclusion, therefore, of this investigation is that there is no evidence for the existence of complexation between the parabens and urea and between amino acids and urea. It is sufficient to consider bulk solvent properties to account for the variation in solubilities with urea concentration. Such an explanation, and the associated absence of thermochemical proof for the existence of complexes between the study solutes and urea, is consistent with the developing theories of the hydrophobic effect.

The data reported here and its analysis support the view that the role of urea in aqueous solutions of apolar substances is due to urea's capacity of insertion into the solvation shell, displacing water molecules and leading to an overall favourable entropy change in relation to solution in water.

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