THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Mechanism of Hydrophobic Drug Solubilization by Small Molecule Hydrotropes

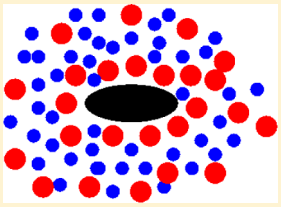
Jonathan J. Booth,[†,‡] Steven Abbott,[§] and Seishi Shimizu*[,‡]

[‡]York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom

[§]Steven Abbott TCNF Ltd., 7 Elsmere Road, Ipswich, Suffolk IP1 3SZ, United Kingdom

Supporting Information

ABSTRACT: Drugs that are poorly soluble in water can be solubilized by the addition of hydrotropes. Albeit known for almost a century, how they work at a molecular basis is still controversial due to the lack of a rigorous theoretical basis. To clear up this situation, a combination of experimental data and Fluctuation Theory of Solutions (FTS) has been employed; information on the interactions between all the molecular species present in the solution has been evaluated directly. FTS has identified two major factors of hydrotrope-induced solubilization: preferential hydrotrope−solute interaction and water activity depression. The former is dominated by hydrotrope−solute association, and the latter is enhanced by ionic dissociation and hindered by the self-aggregation of the hydrotropes. Moreover, in stark contrast to previous hypotheses, neither the change of solute hydration nor the water structure accounts for hydrotropy. Indeed, the rigorous FTS poses serious doubts over the other common hypothesis: self-aggregation of the hydrotrope hinders, rather than promotes, solubilization.



1. INTRODUCTION

Poor solubility of drug molecules poses a serious problem in designing novel drugs. Yet this problem has often been overcome by formulating the drug with nontoxic, water-soluble molecules, commonly called hydrotropes.[1−3] By the use of the hydrotropes, drug solubility can be increased by several orders of magnitude.[1−3] The terms hydrotrope and solubilizer are used to cover a confusing number of methods to increase solubility.[1−4] In this paper the term is reserved for the effects of high concentrations (up to 3 M) of small molecules such as urea. Future papers will aim to understand some of the other effects under the terminology of "hydrotrope" or "solubilizer".

What is the mechanism by which the hydrotropes increase the solubility of hydrophobic solutes? Despite almost a century of investigations,[1−4] the literature is still full of conflicting views on the origin of hydrotropy which can be classified into the following three categories: (i) self-assembly of hydrotrope molecules; (ii) disruption of water structure by the hydrotropes; (iii) formation of solute−hydrotrope complexes.

To clarify the true origin of the hydrotropy at a molecular basis, it is necessary to ask why there are three different answers to the same question; are they three different facets of the same phenomenon, or are some of the above hypotheses erroneous? To answer these questions, let us first summarize briefly the origin and scope of these hypotheses.

Self-Aggregation Hypothesis. The self-aggregation hypothesis assumes that the hydrotropes form clusters or aggregates in the bulk solution. It is this hydrotrope clustering that promotes the solubility of the drugs because the solutes can be accommodated inside the cluster.[5−8] This scenario is parallel to the solubilization by micelles. Indeed, solubilization increases only after a certain hydrotrope concentration (called the minimum hydrotrope concentration),[5−8] which suggests a resemblance to the critical micelle concentration. This scenario, however, is not likely for amphiphilic molecules with short chains (or no chains in the case of urea), which are unlikely to form micellar structures.[2,9] There is room, then, to question whether self-aggregation is truly and universally the driving force of hydrotropy.

Water Structure Hypothesis. The water structure hypothesis is based on an assumption that the hydrotropes do not bind directly to the solutes.[10] Consequently, the effect of the hydrotropes on solute−water interaction must be indirect.[10] Frank and Franks articulated this view via a simple lattice model of solution; urea breaks the structure of water, thereby counteracting "iceberg" formation (namely, the enhancement of the water structure and the decrease of entropy as a consequence) around the hydrophobic solutes, which is the cause (according to Frank and Evans[9]) of the hydrophobic effect.[6,10,11] The lack of change in the solute UV/vis spectrum in the presence of urea and nicotinamide supports this scenario.[10]

However, a number of difficulties confront this hypothesis. First, the very definition of the water structure is ambiguous.[12] This is because the "water structure" has been formulated upon a solution model as crude as lattice theory.[10,13] Second, not only have the existence and nature of the "iceberg" been under controversy, but also whether the "iceberg" really drives the hydrophobic hydration has been questioned.[14,15] In addition, a rigorous statistical thermodynamic analysis in the context of proteins has shown that the change of hydrophobic hydration induced by urea is negligibly small compared to the

Received: October 4, 2012
Revised: November 21, 2012
Published: December 13, 2012

accumulation of urea around the hydrophobic regions.[12] Thus, the basis of the water structure hypothesis has been shown also to be questionable.

Solute−Hydrotrope Complexation Hypothesis. The solute−hydrotrope complexation hypothesis claims low stoichiometry complexes (such as 1:1 or 1:2) as the origin of hydrotropy.[16,17] This scenario has been supported by computational studies for the caffeine−riboflavin system,[17] as well as by structural chemistry data for nicotiamides via the possibility of solute−hydrotrope π-stacking.[16,17] However, the lack of change in the riboflavin UV/vis spectrum contradicts this hypothesis.[10] Yet recent, rigorous statistical thermodynamic analyses have demonstrated that subtle cosolvent accumulation around the solute, rather than stoichiometric binding, can significantly increase solubility.[18−26] This hypothesis therefore needs to be reformulated in the framework of a rigorous statistical thermodynamic theory before its validity can be evaluated.

We have thus seen that all of the above hypotheses suffer from the lack of a rigorous statistical thermodynamic basis. Moreover, few studies have systematically analyzed the solubilizing capability across a range of hydrotropes and solutes in order to reach a universal generalized molecular-based understanding. To overcome such shortcomings, this paper provides a rigorous statistical thermodynamic approach to hydrotropy and analysis of experimental data within its framework. In particular, the Fluctuation Theory of Solution (FTS), an exact statistical thermodynamic theory,[15,18−27] has been employed, which can provide information regarding the statistical interactions between all the molecular species in solution, solely from basic thermodynamic data, such as density, osmotic pressure, and solubility.[18−27] Based on this rigorous foundation, the true molecular origin of hydrotropy will be investigated in the following.

2. THEORY

Consider a three component solution consisting of water (i = 1), solute (i = 2), and hydrotrope (i = 3) molecules. The solute is at the infinite dilution limit, an approximation appropriate and well-established for low-solubility systems.[12,15,18−27] The central question of this paper is to understand, at a microscopic level, the decrease of hydration-free energy of the solute, μ_2^*, upon the addition of hydrotropes. This is quantified by the change of μ_2^* with respect to the molarity of the hydrotrope, n_3: $(\partial\mu_2^*/\partial n_3)^{1/2}_{T,P,n_{2\to 0}}$. This differential, which will be called the ΔG gradient throughout this paper, has a clear physical interpretation. FTS, an exact classical statistical thermodynamic theory, connects the ΔG gradient to the radial distribution functions between the species.[18−27] To carry out this connection, it is helpful to decompose this ΔG gradient into the following two thermodynamic quantities, whose molecular interpretation has been well-established by the FTS:

$$\left(\frac{\partial\mu_2^*}{\partial n_3}\right)_{T,P,n_2\to 0} = \left[-\left(\frac{\partial\mu_2^*}{\partial\mu_1}\right)_{T,P,n_2\to 0}\right]\left[-\left(\frac{\partial\mu_1^*}{\partial n_3}\right)_{T,P,n_2\to 0}\right] \tag{1}$$

The first contribution in eq 1, $-(\partial\mu_2^*/\partial\mu_1)^{1/2}_{T,P,n_{2\to 0}} \equiv \nu_{21}$, is the chemical potential-based preferential hydration parameter.[19,21,22,25] This term, according to the FTS, can be expressed in the following:[18−27]

$$\nu_{21} \equiv -\left(\frac{\partial\mu_2^*}{\partial\mu_1}\right)_{T,P,n_2\to 0} = n_1(G_{21} - G_{23}) \tag{2}$$

where n_1 is the molarity of water. More importantly, G_{ij}, commonly called the Kirkwood−Buff (KB) parameter, is defined through the radial distribution function, g_{ij}, as:[18−27]

$$G_{ij} = 4\pi\int_\infty^0 [g_{ij}(r) - 1]r^2 dr \tag{3}$$

in which r is the distance between the centers of the molecular species i and j. Thus, the preferential hydration parameter, according to eq 2, signifies the net excess solute−water distribution G_{21} over the solute−hydrotrope interaction G_{23}.[18−27]

These solute−solvent KB parameters, G_{21} and G_{23}, can be determined entirely from the experimental data.[18−27] This is done by solving a set of simultaneous equations which consist of eq 2, as well as of another relationship, independent of eq 2, which connects G_{21} and G_{23}. Such a relationship is the partial molar volume of the solute, V_2:

$$V_2 = -V_1 n_1 G_{21} - V_3 n_3 G_{23} + RT\kappa_T \tag{4}$$

where R is the gas constant and κ_T is the coefficient of isothermal compressibility of the bulk solution. Solute−water and solute−hydrotrope KB parameters (G_{21} and G_{23}) can, thus, be determined experimentally through eqs 2 and 4.

Let us now turn to the second contribution in eq 1, $-(\partial\mu_1^*/\partial n_3)^{1/2}_{T,P,n_{2\to 0}}$. This term signifies the water activity depression as the hydrotrope is added. This contribution also has a microscopic interpretation in terms of KB parameters:[27]

$$-\left(\frac{\partial\mu_1}{\partial n_3}\right)_{T,P,n_2\to 0} = \frac{RT}{n_1(1 + n_3G_{33} - n_3G_{13})} \tag{5}$$

The water activity depression is, thus, related to hydrotrope−hydrotrope $G_{33}$and hydrotrope−water G_{13} interactions, in stark contrast with the popular expectation that water activity reflects the "water structure" in the solution.

The KB parameters which are responsible for the water activity depression (G_{33} and G_{13}), as well as water−water KB parameter G_{11}, can also be determined entirely from the experimental data through the following equation:[15,27]

$$G_{ii} = RT\kappa_T - \frac{1}{n_i} + \frac{n_jV_j^2(n_i + n_j)}{n_iD},$$
$$G_{ij} = RT\kappa_T - \frac{V_iV_j(n_i + n_j)}{D} \tag{6}$$

where V_i is the partial molar volume of the species i, which can readily be calculated from the composition-dependent density data of the mixture. The factor D in eq 6 is defined as[15,27]

$$D = x_1\left(\frac{\partial\ln(a_1)}{\partial x_1}\right)_{T,P} = x_3\left(\frac{\partial\ln(a_3)}{\partial x_3}\right)_{T,P} \tag{7}$$

where a_i is the activity of the species i, which can be measured from osmometry, and x_i is the mole fraction. Not only the solute−solvent, but also the solvent−solvent KB parameters can be determined completely experimentally.

In applying the FTS to electrolyte hydrotropes, cations, and anions have been assumed to be treated as single species as a

whole, as in the previous KB approaches to ionic solutions.[21] Therefore, any G_{ij} reported for the salt hydrotropes represents the overall value for the salt involved therein. This has an important consequence. For a hydrotrope dissociating in water into ν ions, eq 1 is rewritten as

$$\left(\frac{\partial\mu_2^*}{\partial n_{3,\mathrm{salt}}}\right)_{T,P,n_2\to 0} = \left[-\left(\frac{\partial\mu_2^*}{\partial\mu_1}\right)_{T,P,n_2\to 0}\right] \left[-\nu\left(\frac{\partial\mu_1^*}{\partial n_{3,\mathrm{ion}}}\right)_{T,P,n_2\to 0}\right] \quad (8)$$

Here $n_{3,\mathrm{salt}}$ signifies the salt concentration, $n_{3,\mathrm{ion}} = \nu n_{3,\mathrm{salt}}$ denotes the ion concentration in the solution. Throughout this paper, ΔG slope for the electrolytes is $(\partial\mu_2^*/\partial n_{3,\mathrm{salt}})^{1/2}_{T,P,n_{2\to 0}}$, whereas the water activity depression, which can be calculated from ion−ion and ion−water KB parameters (G_{33} and G_{13}), is $-(\partial\mu_1^*/\partial n_{3,\mathrm{ion}})^{1/2}_{T,P,n_{2\to 0}}$, whose important consequence will be discussed below.

In summary, information regarding molecular interactions in the water−solute−hydrotrope mixture is accessible by thermodynamic measurements; rigorous statistical thermodynamic theory then provides a connection between thermodynamic data and the solution structure at a molecular level.

3. EXPERIMENTAL SECTION

Two hydrotropes (sodium benzoate [sb] and sodium salicylate [sa]) and two solutes (butyl acetate [BA] and benzyl benzoate [BB]) were purchased from Sigma Aldrich; urea was purchased from Fischer. All these substances, whose purity was at least 99%, have been used without further purification. Solutions were made up using distilled, filtered, and deionized water, which was then degassed. Masses were measured using a four figure balance accurate to 0.0001 g.

KB parameters (eq 3) quantify all the interactions between the molecular species in the solution. To obtain solute−water and solute−hydrotrope KB parameters from eqs 2 and 4, solubility, osmotic, and density data are necessary.[18−27]

Solubility data at 303 K have been taken from Gandhi and co-workers.[28,29] A well-established procedure has been followed in the experimental evaluation of μ_2^*.[30,31] Because μ_2^* will be differentiated with respect to n_1 or μ_1 (eqs 1 and 2), we can choose μ_2^* for pure water (which does not depend on n_1 or μ_1). Thus, we use the solubility expressed in molarity concentration, n_2, namely, $\mu_2^* = -RT\ln(n_2/n_2^0)$, where n_2^0 is the solubility of the drugs in pure water.[27] Figure 1 shows the dependence of μ_2^* against the molarity of the hydrotropes.

To calculate the preferential hydration parameter, it is necessary to plot μ_2^* against $-RT\ln a_1$ (Figure 2); its gradient yields the preferential hydration parameter.[18−27] Water activity data in the aqueous urea have been taken from Stokes,[32] whereas those in aqueous sodium benzoate and sodium salicylate have been measured at 303 K using a Gonotec Osmomat 070 vapor pressure osmometer. Our data, which are summarized in Table 1, are in close agreement with those published at 298 K.[33] The fitting using the molality concentration follows a well-established method of calculation.[32,34]

Density data for aqueous urea solutions have been taken from Stokes,[30] whereas those for aqueous sodium benzoate and sodium salicylate solutions have been measured using an Anton Paar DSA 5000 density meter. The results are summarized in Table 2. The density data have then been converted to the partial molar volumes, V_1 and V_3, through a well-established method.[27,35]

Measuring partial molar volume of the solutes (V_2) as a function of hydrotrope concentration (n_3) poses difficulty, due

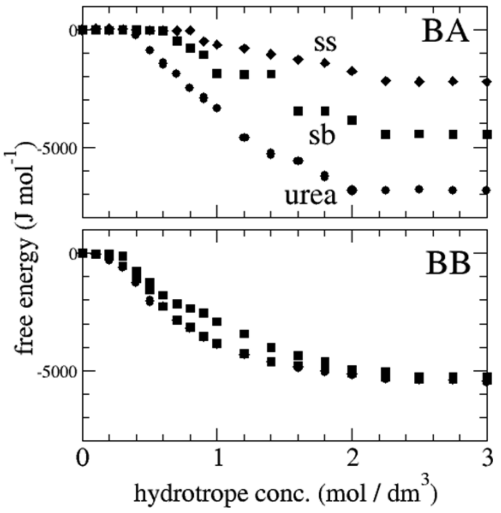


Figure 1. Dependence of the transfer free energy, μ_2^*, on the hydrotrope concentration, for the solute BA (top) and BB (bottom). The effect of hydrotropes on solvation ΔG differs widely for the solute BA, little for BB. The solubility data has been taken from refs 28 and 29.

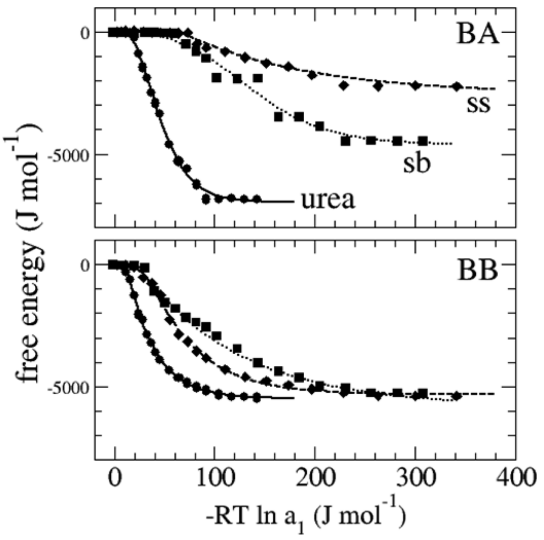


Figure 2. Dependence of transfer free energy, μ_2^*, on $-RT\ln a_1$ (a_1 is the water activity), for the solutes BA (top) and BB (bottom). Preferential hydration parameter, according to eq 2, is the gradient of this graph.

Table 1. Regression Coefficients for the Dependence of Osmolality against Molality m_3 of the Hydrotropes[a]

hydrotrope	a	b	c	R^2
urea	−0.02047	0.975	5.044×10^{-3}	1.000
sodium benzoate	−0.1396	2.192	-1.605×10^{-2}	0.997
sodium salicylate	0.000	1.714	9.259×10^{-2}	0.996

[a]The fitting equation is of the form: osmolality = $am_3^2 + bm_3 + c$.

dx.doi.org/10.1021/jp309819r | J. Phys. Chem. B 2012, 116, 14915−14921

Table 2. Regression Coefficients and the R^2 Values for the Density ρ of the Aqueous Hydrotrope Solutions[a]

	a	b	c	R^2
u	-8.711×10^{-5}	1.559×10^{-2}	0.9957	0.999
sb	-1.704×10^{-3}	5.726×10^{-2}	0.9956	0.999
ss	-1.352×10^{-3}	6.560×10^{-2}	0.9955	0.999

[a]u, urea; sb, sodium benzoate; ss, sodium salicylate. The fitting equation is in the following form: $\rho = an_3^2 + bn_3 + c$. The raw data is found in the Supporting Information (Table S2).

to the low solubility of the solutes before the maximum solubilization has been reached. However, as we have shown previously that, in the calculation of G21 and G23, the contribution of V_2 is much smaller compared to $V_1\nu_{21}$, and may therefore be omitted in the calculation.[20,21] Therefore, minor changes of the value of V2 caused by the presence of cosolvents does not affect the calculated KB parameters. Indeed, as Chalikian and co-worker have demonstrated, the change of partial molar volumes against cosolvent concentration is small.[35,36] V_2 has therefore been calculated from the density change upon the addition of the solutes at 3 M hydrotrope concentration, as has been described by Chalikian and co-workers.[35,36] The solutions were stirred overnight to ensure complete dissolution of the solute. The resulting partial molar volumes are shown in Table 3.

Table 3. Partial Molar Volumes of the Solutes in the 3M Aqueous Hydrotrope Solution, Calculated from the Density Data[a]

cosolvent	solute	density/g cm^{-3}	density with solute/g cm^{-3}	V_2/cm^3 mol^{-1}
urea	butyl acetate	1.03439	1.03438	112.30
urea	benzyl benzoate	1.03439	1.03437	205.20
sodium benzoate	butyl acetate	1.13059	1.12944	103.23
sodium benzoate	benzyl benzoate	1.13059	1.12955	189.18
sodium salicylate	butyl acetate	1.13538	1.13511	102.54
sodium salicylate	benzyl benzoate	1.13538	1.13492	187.42

[a]See Chalikian[35,36] for the calculation procedure.

The experimental data gathered above are sufficient also to calculate G_{11}, G_{13}, and G_{33} by the use of eqs 6 and 7. For this, the activity coefficients of the hydrotropes, γ_3, which have been calculated from Table 1 through standard thermodynamic procedure,[27,37,38] have been fitted against the mole fraction (Table 4).

Table 4. Regression Coefficients for the Activity Coefficient of the Hydrotropes against the Mole Fraction of the Hydrotropes x_3[a]

hydrotrope	a	b	c
urea	0.988	−3.2172	0.998967
sodium benzoate	4.76454	−41.8332	−180.91
sodium salicylate	4.79822	−181.988	261.377

[a]Note that for electrolyte hydrotropes, the mole fraction refers to that of the ions. The equation used is of the form $\gamma_3 = 1 + ax_3 + bx_3^2 + cx_3^3$.

Finally, the fitting coefficients used to fit the hydration-free energy to the water activity, which is necessary to calculate the preferential hydration parameters (eqs 1 and 2) are shown in Table 5.

Table 5. Regression Coefficients for the Transfer Free Energy as a Function of $-RT \ln a_1$ (see Figure 2)[a]

system	a	b	c	x_0	y_0	R^2
u + BA	−7118	19.860	363200	−216.8	143	0.997
u + BB	5492	−2.513	0.091	12.21	−5490	0.999
sb + BA	4910	−36.300	0.802	119.90	−4630	0.981
sb + BB	5813	−0.702	0.007	23.11	−5823	0.995
ss + BA	2572	−2.560	0.020	64.99	−2570	0.989
ss + BB	5310	−6.314	0.132	30.75	−5324	0.997

[a]The fitting equation is: $\mu_2^* = y_0 + a/[1 + e - ((-RT \ln(a_1) - x_0)/(b)]c$. Abbreviation: BA, butyl acetate; BB, benzyl benzoate; sb, sodium benzoate; ss, sodium salicylate; u, urea.

4. RESULTS AND DISCUSSION

What are the driving forces of hydrotropy? To answer this question, we must understand the change in the molecular interactions when the hydrotropes are added. Yet each change in the interaction should clearly be connected to thermodynamics. FTS for the first time has made this connection rigorously at a molecular level, allowing hydrotropy to be characterized by structural thermodynamics.

The systems we have chosen pose challenges to the traditional hypotheses on the mechanism of hydrotropy. First, although three hydrotropes (urea, sb, and ss) exhibit different solubilization of BA (Figure 1, top), the same hydrotropes show similar solubilization of BB (Figure 1, bottom). If the hydrotropy were driven chiefly by the properties of bulk solution, such as hydrotrope self-aggregation or the hydrotrope-induced change of the water structure, BA and BB should exhibit similar behavior with regards to solubilization. What, then, makes the same set of hydrotropes act so differently for these solutes? We will answer this question from preferential hydration and water activity depression, both analyzed at a molecular level.

Hydrotropes Accumulate around the Solutes. The first contribution to the ΔG gradient is the preferential hydration parameter (eq 1). In the hydrotrope concentration range in which solubility increases, the preferential hydration parameter is negative for all the systems (Figure 3). The negative preferential hydration is the consequence of $G_{23} > G_{21}$ (see eq 2), namely, the hydrotropes accumulate more around the solutes than water. This has traditionally been termed the "preferential interaction" of the hydrotropes with the solutes.[38,39]

This preferential solute−hydrotrope interaction is only relative; that is to say, it alone cannot tell whether it is due to the accumulation of the hydrotropes around the solute or to the depletion of water around the solutes induced by the hydrotropes.[19,39] To establish the correct scenario, both G_{21} and G_{23} must be calculated.[18−27] This is done by solving eqs 2 and 4, which shows that the change in the preferential hydration parameter comes dominantly from the change of solute−hydrotrope interaction, $-n_1G_{23}$ (Figure 3); the contribution from the change of hydration is minor: the larger in magnitude the preferential hydration parameter, the more dominant $-n_1G_{23}$. Here, the negative sign of $-n_1G_{23}$, or the

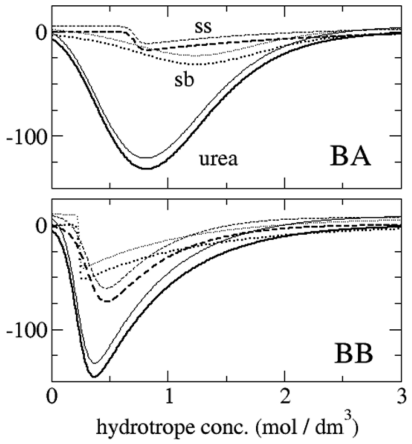


Figure 3. Plot of prefential hydration parameters (thick line), compared with the solute−hydrotrope interaction, $-n_1G_{23}$ (thin lines), for the solutes BA (top) and BB, in the presence of the hydrotropes. Solute−hydrotrope interaction, $-(n_1/n_3)G_{23}$, is seen to contribute increasingly dominantly as the preferential hydration parameter becomes more negative.

positive sign of G_{23}, shows that the hydrotropes do accumulate around the solutes. This shows that it is the accumulation of the hydrotropes around the solute, which is the dominant driving force of the preferential hydrotrope−solute interaction.

Urea, in particular, accumulates most strongly among the three hydrotropes studied here. This may seem to be in contradiction with the fact that, while urea is the strongest hydrotrope for BA, its effectiveness for BB is only comparable to the others. This apparent contradiction can be resolved by considering the second contribution to the ΔG slope: water activity depression, which will be discussed after a consideration of hydration changes.

Hydration Change Induced by the Hydrotropes Is a Minor Contribution. The dominance of hydrotrope−solute association in the preferential hydrotrope−solute interaction, as shown above, has another important implication: that the contribution of solute hydration, n_1G_{21} is minor (as can easily been seen from eq 2). Indeed, compared to the changes in $-n_1G_{23}$ with respect to the concentration and hydrotrope species, the change of n_1G_{21} is minor. This is contrary to the water structure hypothesis, which has attributed the origin of hydrotropy to the solute's hydration change in the presence of the hydrotropes.[10,11]

Indeed, as we have seen in the Introduction, the basic assumption of the water structure hypothesis, articulated by Frank and Franks, is that the hydrotrope (in their particular case, urea) does not "interact directly" with the solutes; it affects the hydration instead, through the breaking of the water structure.[9]

It is this assumption that is in stark contradiction to our calculation (Figure 3): preferential interaction is not the consequence of the hydration change, but of the solute−hydrotrope interaction. Such a solute−hydrotrope interaction is often weak and nonspecific, rather than stoichiometric,[19−26,39,40] consistent with the lack of spectroscopic evidence for complexation.[10] Nevertheless, these weak interactions have a significant effect. The hydrotrope−solute complexation hypothesis should therefore be revised to incorporate weak, nonspecific interactions.

We have focused on moderate hydrotrope concentrations (3 M). In the higher hydrotrope concentrations, the solute−water interaction may be affected by the presence of the hydrotropes. Whether the hydration contribution becomes significant in the higher concentration range of the hydrotropes should await further investigations.

Water Activity Depression: 1. Electrolytic Dissociation Enhances Hydrotropy. The second contribution to the ΔG slope, necessary for rationalizing the different hydrotropy for the two solutes, is water-activity depression. This contribution has been calculated for all the system using eqs 5−7. What is striking is the water activity depression at low hydrotrope concentration: for sa and sb it is twice as large as for urea (Figure 4a). This has arisen merely because of the

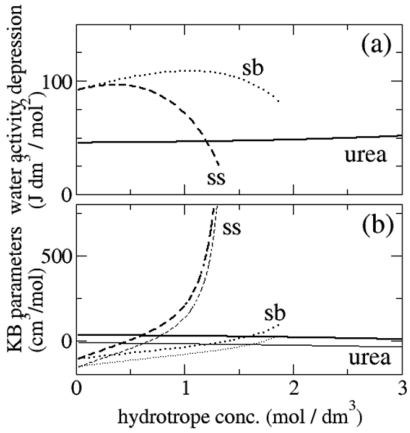


Figure 4. (a) Dependence of water activity depression (as introduced in eq 1), $-(\partial\mu_1^*)/(\partial n_3)_{T,P,n_{2\to 0}}$, on the hydrotrope concentration. (b) $G_{33} - G_{13}$ (thick lines), whose reciprocal determines the water activity depression, against the hydrotrope concentration. Its comparison with the hydrotrope aggregation KB parameter, G_1 (thin lines), shows that hydrotrope aggregation prevents water activity depression.

concentration scale of the experiment: hydrotrope concentration usually refers to the salt concentration rather than that of the constituent ions. This can be rationalized by the use of eqs 5 and 8. At the limit $n_3 \to 0$, eq 5 (applied to the ions) reduces to $-(\partial\mu_1)/(\partial n_{3,\mathrm{ion}})_{T,P} = RT/n_1^0$, where n_1^0 is the density of pure water. Water activity depression, in this limit, is $\nu(RT)/(n_1^0)$. Hence, water activity depression in sa and sb ($\nu = 2$) is twice as large as in urea (no dissociation, i.e., $\nu = 1$).

What is remarkable here is that it is this electrolyte dissociation that closes the gap of preferential hydrotrope−solute interaction between urea and the salts; in the case of BB, it causes the close ΔG slope between urea and the electrolyte hydrotropes. Thus, electrolyte dissociation promotes solubilization through water activity depression.

Water Activity Depression: 2. Self-Association of Hydrotropes Prevents Solubilization. If the dissociation of the hydrotropes promotes solubilization, does the self-aggregation prohibit solubilization? This question can be answered most strikingly by the case of SS, which exhibits the most dramatic increase of G_{33} (KB parameter for hydrotrope−hydrotrope interaction) among the three, as seen in Figure 4b. Consequently (see eq 5), the water activity depression (which increases solubilization) for SS reduces rapidly as the concentration goes up (Figure 4a), making ss a poor hydrotrope for BA. We have thus arrived at a startling scenario: hydrotrope self-aggregation hinders hydrotropy.

This scenario is in stark contrast with the traditional view. The first reason is that self-aggregation has long been

considered to be the major driving force of hydrotropy, having been inspired by an analogy to miceller solubilization,[5−7] despite suggestions that many hydrotropes have side chains too short to form micelles.[10] In contrast to the phenomenological basis of the traditional view, FTS is a rigorous and exact theory, where the increase of G_{33} (the dominant contribution to the increase of $G_{33} - G_{13}$) and the reduction in the water activity depression are connected mathematically by eq 5. FTS thus provides a theoretical justification toward the observation that self-aggregation does not promote solubilization. This is in agreement with a recent study in the context of protein solvation.[41] The second reason is that hydrotrope self-aggregation is the cause of a reduction in water activity depression, in spite of the popular view that water activity must somehow be related to the "water structure".[42]

Change of "Water Structure" Cannot Rationalize Solubilization. FTS has already shown, contrary to the water structure-based hypothesis,[9,11] that the hydrotropes associate with the solutes and that the hydrotrope-induced hydration change contributes little to solubilization. FTS has already provided evidence against the basic assumption of the water structure hypothesis, that there is no hydrotrope-solute association and that the hydrotropes consequently influence indirectly on solute hydration. Important questions, however, remain unanswered. Do hydrotropes really break the water structure? Consequently, does the water structure breaking have any correlation with solubilization?

The difficulty in addressing these questions comes directly from the difficulty in defining what "water structure" or its breaking really means at a molecular level.[27,42−44] Consequently, the difficulty has been acknowledged in the literature in establishing a clear relationship between water structure and the radial distribution function (and G_{11}).[27,28,43,44]

Yet we believe that whether there is any correlation between solubilization and the *change* of water structure can be addressed, by comparing the ΔG slope with the change of G_{11}. One would expect, according to the water structure hypothesis, that urea, the most effective hydrotrope, has the strongest effect on "water structure" and hence on G_{11}. Yet G_{11} for urea, according to the calculation, is affected the least among the three (Figure 5). This conclusion does not depend on the whether water structure breaking corresponds to the increase or decrease of G_{11}. This provides yet further evidence against the water structure hypothesis, in agreement with the already-published scepticism.[12,45]

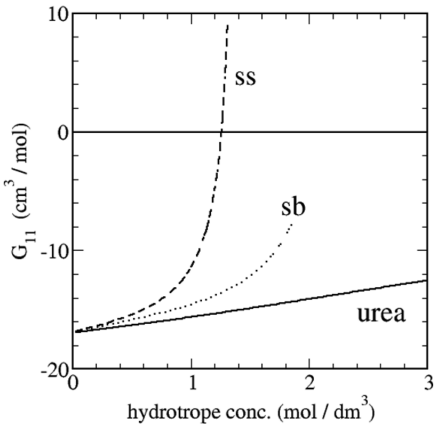


Figure 5. Water−water interaction KB parameter, G_{11}, against the hydrotrope concentration, in the presence of urea (solid), ss (dashed), and sb (dotted).

Origins of the Minimum Hydrotrope Concentration. As mentioned earlier, the fact that there is a minimum hydrotrope concentration has led to a false analogy with the critical micelle concentration and, therefore, the notion that hydrotrope self-aggregation was critical for solubilization. Indeed, many hydrotropes have been demonstrated not to form micelles.[8] Given that the dominant driver for solubilization is solute−hydrotrope association, how is the critical hydrotrope concentration explained?

A clue toward the answer comes from the study of protein denaturants. The origin of the sigmoidal behavior of the hydration free energy against the denaturant concentration, similar to Figure 2, has been attributed to the cooperativity in the binding of denaturants to proteins.[46] We propose here that hydrotropes, too, bind cooperatively to the solutes. Minimum hydrotrope concentration is thus the consequence of cooperative hydrotrope−solute binding, instead of a phenomenon reminiscent of critical micelle concentration.

The existence of minimum hydrotrope concentration, as well as the saturation of solubilization, is the consequences of the existence of minima in the preferential hydration parameter, as is obvious from eq 1. This intriguing behavior of preferential hydration, especially for urea and sb, may be rationalized also by the cooperative hydrotrope−solute binding. To address this question fully, molecular simulation is indispensable.

5. CONCLUSION

How hydrophobic drug molecules can be solubilized through adding hydrotropes has been elucidated at a molecular basis, within a rigorous theoretical framework of the Fluctuation Theory of Solutions (FTS). In this framework, information regarding the interaction between all the molecular species present in the system can be obtained solely from thermodynamic data (such as solubility, osmotic coefficients, and density). Such a combination of theory and experiments has paved the way to clear up the long-lasting controversy regarding the origin of the type of hydrotropy discussed here.[1−9,11,17,18]

We have identified two major driving forces which promote hydrotrope-induced solubilization: the first is the solute−hydrotrope binding, the second is water activity depression. There are two main contributions to the second, ionic dissociation (which increases (doubles) the water activity depression) and the self-aggregation of the hydrotropes (which decreases the water activity depression). Hydrotropy can thus be explained by the combination of these two factors.

This conclusion is in stark contrast with the popular hypotheses. First, self-aggregation has long been considered as a factor which promotes solubilization.[5−7] The analysis here shows the contrary; self-aggregation hinders the solubilization through the decrease of water activity depression. Second, against the popular assumption,[10−13] neither the water structure change in the presence of the hydrotropes nor the change in the hydration of the solute has a significant effect on the solubilization. The rigorous FTS thus has effectively demolished these long-held assumptions on the origin of hydrotropy.

This study has thus identified the major driving forces of hydrotropy. With some simplifications (e.g., through the use of approximations to V_2) it should be possible to perform the same FTS analysis on many more classical hydrotrope systems

(e.g., nicotinamide) for which solubility curves are available. Yet there is still a long way to go toward the rational design of the hydrotrope required for a given drug, as well as toward the optimization of the hydrotrope's chemical structures. This is because all the interactions analyzed in this paper are by definition at the level of the potentials of mean force.[19−28] At such a level, the contribution of the water molecules is only implicit. A fuller understanding of these effects will have to be based on explicitly molecular approaches, such as molecular dynamics or Monte Carlo simulations. Nevertheless, FTS employed in this study has provided a guideline toward such future studies, through establishing a clear connection between molecular interactions, and the thermodynamics of solubilization.

Given that there are many other uses of the words "hydrotrope" and "solubilizer" it is hoped that the FTS approach can be used to throw light on other cases which, from the literature, seem to rely for explanations more on phenomenology than on rational analysis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The raw density and osmotic data measured for the sodium salts. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +44 1904 328281. Fax: +44 1904 328266. E-mail: seishi.shimizu@york.ac.uk.

Present Address

†School of Chemistry, University of Leeds, Leeds, LS2, 9JT, U.K.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Neuberg, C. *Biochem. Z.* **1916**, *76*, 107−176.
(2) (a) Hodgdon, T. K.; Kaler, E. W. *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 121−128. (b) Subbarao, C. V.; Chackravarthy, I. P. K.; (c) Sai Bharadwaj, A. V. S. L.; Prasad, K. M. M. *Chem. Eng. Technol.* **2012**, *35*, 225−237.
(3) Saleh, A. M.; Elkhordagui, L. K. *Int. J. Pharm.* **1985**, *24*, 231−238.
(4) Cui, Y.; Xing, C.; Ran, Y. *J. Pharm. Sci.* **2010**, *99*, 3048−59.
(5) Coffman, R. E.; Kildsig, D. O. *Pharm. Res.* **1996**, *13*, 1460−1463.
(6) Kim, J. Y.; Kim, S.; Papp, M.; Park, K.; Pinal, R. *J. Pharm. Sci.* **2010**, *99*, 3953−3965.
(7) Balasubramanian, D.; Srinivas, V.; Gaikar, V. G.; Sharma, M. M. *J. Phys. Chem.* **1989**, *93*, 3865−3870.
(8) Bauduin, P.; Renoncourt, A.; Kopf, S.; Touraud, D.; Kunz, W. *Langmuir* **2005**, *21*, 6769−6775.
(9) Frank, H. S.; Franks, F. *J. Chem. Phys.* **1968**, *48*, 4746−4757.
(10) Coffman, R. E.; Kildsig, D. O. *J. Pharm. Sci.* **1996**, *85*, 951−954.
(11) Frank, H. S.; Evans, M. W. *J. Chem. Phys.* **1945**, *13*, 507−532.
(12) (a) Shimizu, S. *Chem. Phys. Lett.* **2011**, *517*, 76−79. (b) Shimizu, S. *Chem. Phys. Lett.* **2011**, *514*, 156−158.
(13) Graziano, G. *Chem. Phys. Lett.* **2009**, *483*, 67−71.
(14) Dill, K. A.; Truskett, T. M.; Vlachy, V.; Hribar-Lee, B. *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 173−200.
(15) Ben-Naim, A. *Molecular Theory of Water and Aqueous Solutions*; World Scientific: Singapore, 2011.
(16) Sanghi, R.; Evans, D.; Yalkowsky, S. H. *Int. J. Pharm.* **2007**, *336*, 35−41.
(17) Cui, Y. *Int. J. Pharm.* **2010**, *397*, 36−43.
(18) Shimizu, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 1195−1199.
(19) Shimizu, S.; Boon, C. L. *J. Chem. Phys.* **2004**, *121*, 9147−55.
(20) Shimizu, S.; McLaren, W. M.; Matubayasi, N. *J. Chem. Phys.* **2006**, *124*, 234905.
(21) Smith, P. E. *J. Phys. Chem. B* **2004**, *108*, 18716−18724.
(22) Smith, P. E. *J. Phys. Chem. B* **2006**, *110*, 2862−2868.
(23) Smith, P. E.; Mazo, R. M. *J. Phys. Chem. B* **2006**, *112*, 7875−7884.
(24) Shulgin, I. L.; Ruckenstein, E. *J. Phys. Chem. B* **2006**, *110*, 12707−12713.
(25) Ruckenstein, E.; Shulgin, I. L. *Adv. Colloid Interface Sci.* **2006**, *123−126*, 97−103.
(26) Ben-Naim, A. *Molecular Theory of Solutions*; Oxford University Press: New York, 2006.
(27) Chitra, R.; Smith, P. E. *J. Phys. Chem. B* **2002**, *106*, 1491−1500.
(28) Gandhi, N. N.; Kumar, M. D.; Sathyamurthy, N. *J. Chem. Eng. Data* **1998**, *43*, 695−699.
(29) Meyyappan, N.; Gandhi, N. N. *J. Chem. Eng. Data* **2005**, *50*, 796−800.
(30) Shimizu, S.; Shimizu, K. *J. Am. Chem. Soc.* **1999**, *121*, 2387−2394.
(31) Chan, H. S.; Dill, K. A. *Annu. Rev. Biophys. Biomol. Str.* **1997**, *26*, 425−459.
(32) Stokes, R. H. *Aust. J. Chem.* **1967**, *20*, 2087−2100.
(33) Ho, P. C.; Bender, T. M.; Kahlow, M. A. *J. Chem. Eng. Data* **1985**, *30*, 292−295.
(34) Gucker, F. T.; Gage, F. W.; Moser, C. E. *J. Am. Chem. Soc.* **1938**, *60*, 2582−2588.
(35) Chalikian, T. V. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 207−235.
(36) Chalikian, T. V. *Biophys. Chem.* **2010**, *156*, 3−12.
(37) Florence, A. T.; Attwood, D. *Physiochemical Principles of Pharmacy*; Pharmaceutical Press: London, 2011.
(38) Timasheff, S. N. *Biochemistry* **2002**, *41*, 13473−13482.
(39) Schellman, J. A. *Q. Rev. Biophys.* **2006**, *38*, 351−361.
(40) Dutkiewicz, E.; Jakubowska, A. *ChemPhysChem* **2002**, *3*, 221−224.
(41) Horinek, D.; Netz, R. R. *J. Phys. Chem. A* **2011**, *115*, 6125−6136.
(42) Ikeguchi, M.; Shimizu, S.; Nakamura, S.; Shimizu, K. *J. Phys. Chem. B* **1998**, *102*, 5891−5898.
(43) Shimizu, S.; Chan, H. S. *J. Chem. Phys.* **2000**, *113*, 4683−4700.
(44) Schellman, J. A. *Biopolymers* **1987**, *26*, 549−559.
(45) Zangi, R. *J. Phys. Chem. B* **2010**, *114*, 643−650.
(46) Klotz, I. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14411−14415.