

# Molecular Association in Solution: A Kirkwood–Buff Analysis of Sodium Chloride, Ammonium Sulfate, Guanidinium Chloride, Urea, and 2,2,2-Trifluoroethanol in Water

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Molecular association has been investigated as a function of solution composition for sodium chloride (NaCl), guanidinium chloride (GdmCl), ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), urea, and 2,2,2-trifluoroethanol (TFE) mixtures with water. A Kirkwood–Buff analysis of the available experimental data provided information concerning the degree of excess association of the various species in solution. The results indicate that increasing concentrations of NaCl have no effect on the excess self-association of water, while GdmCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and urea slightly increase the excess self-association of water. The presence of TFE results in composition dependent effects with a strong increase in the self-association of water at low TFE concentrations. The consequences of these observations in the context of water structure making properties are discussed. The excess self-association of cosolvents (except urea) displayed an initial increase which proceeded through a maximum at low cosolvent concentration and then decreased steadily with concentration. Analysis of the excess solvation of the cosolvents indicated substantially desolvated urea and GdmCl molecules at all concentrations, a constant desolvation of NaCl, and a variation from highly solvated ions at low concentration to zero excess solvation for (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

## Introduction

The addition of solutes or cosolvent molecules to water has a marked effect on the properties of the solution.<sup>1–3</sup> These effects include changes in bulk properties, such as viscosity, surface tension, and dielectric response, as well as changes in molecular properties such as diffusion constants and rotational relaxation times. A common theme in early attempts to understand these effects has been the perturbation of the structure of water by the cosolvent.<sup>1,2,4–9</sup> Hence, cosolvents are often classified in terms of their ability to enhance (water structure makers), or disrupt (water structure breakers), the natural hydrogen bonded structure of liquid water. Water structure breakers are commonly referred to as chaotropes, while water structure makers are referred to as kosmotropes.

Cosolvents also affect the properties of biomolecules in solution. In this context, cosolvents are classified according to their ability to both stabilize the native structure and to decrease the solubility of proteins in solution. The chaotrope and kosmotrope classification is also frequently used to refer to protein structure denaturants and stabilizers, respectively. Somewhat unfortunately, it has become common to interchange the ability of a cosolvent to enhance water structure with an ability to stabilize the structure of a protein, and vice versa. While this simple picture works well for strong structure makers, other cosolvents display more subtle effects, and a variety of factors have to be considered.<sup>1,10</sup>

Urea is a prime example of one of these intermediate cases. Urea solutions are well-known to promote the denaturation of proteins. Urea is therefore regarded as a chaotrope. Early experimental evidence on urea solutions indicated that urea also displayed a chaotropic effect on water structure,<sup>11</sup> although there was also evidence to the contrary.<sup>5,12</sup> The primary reason for

this view resides in early studies of viscosity effects<sup>4</sup> and the ability of urea to enhance the solubility of higher alkanes in water,<sup>13</sup> as described by simple models of alkane solubility.<sup>6</sup> Recently, computer simulation<sup>8,9</sup> and NMR studies<sup>14</sup> have questioned this viewpoint, suggesting that urea may be involved in structure making. It has even been proposed that urea acts as a renaturant for hydrophobic groups.<sup>8</sup>

The real problem lies in trying to interpret the effects of cosolvents on the structure and solubility of biomolecules by reference to the effects of cosolvents on water structure alone. It has been clearly demonstrated that cosolvent effects result from a competition between cosolvent binding to a protein and cosolvent exclusion from the vicinity of a protein.<sup>3</sup> While cosolvent binding to proteins cannot be inferred from studies of cosolvent solutions alone, information concerning cosolvent exclusion, also termed preferential hydration, can sometimes be obtained. However, it is desirable to understand the properties of cosolvent solutions themselves before looking at cosolvent effects on biomolecules. For this reason, and due to the continuing use of water structure making and breaking arguments to understand the properties of simple solutions,<sup>15–19</sup> we have undertaken a study of the properties of several common cosolvents in solution. Our goal is to determine to what extent common cosolvents affect the properties of water and how this may or may not relate to our understanding of cosolvent effects on proteins. In addition, a knowledge of the self-aggregation or association between cosolvent molecules is also of great interest.<sup>20–22</sup>

Here we present the results from a Kirkwood–Buff analysis of the available experimental data for sodium chloride (NaCl), ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), guanidinium chloride (GdmCl), urea, and 2,2,2-trifluoroethanol (TFE) mixtures with water, over a range of concentrations at 300 K and 1 atm, in an attempt to understand the properties of the solutions. These cosolvents represent a series of effects on the structure and stability of

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proteins and peptides. Ammonium sulfate is a precipitant of proteins, NaCl is relatively inert, GdmCl and Urea are denaturants, while TFE is a secondary structure maker. The extent to which these results agree or disagree with the current literature concerning water structure making or breaking is also discussed. Other methods are available to describe association of species (usually salts) in solution.<sup>23,24</sup> The Kirkwood–Buff approach is adopted here, as it does not involve any free parameters and provides a rigorous description of molecular association in solutions.

## Theory

**Kirkwood–Buff Theory.** The Kirkwood–Buff (KB) theory<sup>25–27</sup> of solutions is commonly used to relate integrals over radial distribution functions (rdfs) corresponding to the grand canonical ( $\mu VT$ ) ensemble to macroscopic thermodynamic properties at constant pressure ( $p$ ) and temperature ( $T$ ). The properties are expressed in terms of the KB integrals defined by

$$G_{ij} = 4\pi \int_0^\infty [g_{ij}(r) - 1]r^2 dr \quad (1)$$

where  $g_{ij}(r)$  is the radial distribution function for species  $j$  around species  $i$  in the  $\mu VT$  ensemble. The integrals are symmetric with respect to the interchange of indices  $i$  and  $j$  ( $G_{ij} = G_{ji}$ ), and the quantity  $N_{ij} = \rho_j G_{ij}$  represents the excess or deficit (over a random distribution) of  $j$  molecules in the vicinity of the central  $i$  molecule, where  $\rho_j$  is the number density of species  $j$  in the system. It should be emphasized that KB theory does not make any assumptions concerning the nature or size of the two components or the form of the interaction between them.

For a two-component system consisting of water (w) and a cosolvent (c), the partial molar volumes of the two components,  $\bar{V}_w$  and  $\bar{V}_c$ , the isothermal compressibility of the solution,  $\kappa_T$ , and the derivatives of the mole fraction scale activity coefficients ( $f$ ) can be expressed in terms of the integrals  $G_{ww}$ ,  $G_{cc}$ , and  $G_{cw}$  and the number densities  $\rho_w$  and  $\rho_c$ , of water and cosolvent, respectively.<sup>26</sup> Alternatively, the KB integrals can be determined from experimental quantities (densities, partial molar volumes, compressibilities, and activity coefficient derivatives) using an inversion procedure described previously:<sup>27,28</sup>

$$G_{cw} = RT\kappa_T - \frac{\bar{V}_w \bar{V}_c}{DV_m} \quad (2)$$

$$G_{ww} = G_{cw} + \left(\frac{\bar{V}_c}{D} - V_m\right) \frac{1}{x_w}; \quad G_{cc} = G_{cw} + \left(\frac{\bar{V}_w}{D} - V_m\right) \frac{1}{x_c} \quad (3)$$

where  $D = 1 + x_c(\partial \ln f_c / \partial x_c)_{p,T} = 1 + x_w(\partial \ln f_w / \partial x_w)_{p,T}$ ,  $V_m$  is the molar volume of the solution,  $x_i$  is the mole fraction of species  $i$ , and  $R$  is the gas constant. This is the approach adopted here.

Experimental data at constant temperature and pressure provide KB integrals defined in the  $\mu VT$  ensemble, and hence the relative distribution of the different components under these conditions. This distribution is closely related to the distribution in the  $NpT$  ensemble at the same density and temperature. The assumed similarity between the closed and open system rdfs is based on the fact that the rdfs are primarily determined by the interactions between particles at short range. As pointed out by Ben-Naim,<sup>26</sup> the long-range behavior of the  $\mu VT$  and  $NpT$  rdfs are fundamentally different and differ by a term of the order of  $1/N$  for an ideal gas. Fortunately, this is a negligible quantity

(for large enough  $N$  and away from a phase transition) except when the integration over the rdf extends to infinity.<sup>29</sup> Hence, the open system KB integrals obtained here should provide a good description of associations over the first few molecular diameters in closed systems. This is the correlation region approach of Ben-Naim.<sup>26</sup> Numerical simulation results in closed and open systems at the same density and temperature also support this approximation.<sup>30</sup>

A discussion of the relationships between the KB integrals, the treatment of electrolytes and the properties of reference states can be found in the appendices.

## Methods

**Activity Coefficient Derivatives.** The activity coefficients for both sodium chloride and ammonium sulfate solutions were taken from Robinson and Stokes.<sup>31</sup> The values for the activity coefficients of the inorganic salts have been used repeatedly in the literature, and it is unlikely that there is any significant error in these data. Activity coefficients for the GdmCl solutions were taken from reference 32. The activity coefficients for these solutions were determined from relatively recent calorimetric measurements. The activity coefficients for urea solutions, obtained from heats of dilution, were taken from ref 33. Experimental activity coefficients on the molal scale were converted to those on the mole fraction scale using standard relations.<sup>31</sup> For TFE/water mixtures, excess molar Gibbs energies calculated from vapor pressure measurements as a function of composition were taken from reference 34. In obtaining these data, the authors assumed that the (unknown) second virial coefficient of TFE was identical with that of ethanol.<sup>34</sup>

The logarithms of the activity coefficients were fitted as follows. If the excess molar Gibbs energy is assumed to have the Redlich–Kister form<sup>35</sup>

$$G^{\text{ex}} = x_c(1 - x_c)[A + B(1 - 2x_c) + C(1 - 2x_c)^2 + D(1 - 2x_c)^3 + E(1 - 2x_c)^4] \quad (4)$$

using the relation

$$\left(\frac{\partial \ln f_c}{\partial x_c}\right) = \beta(1 - x_c) \left(\frac{\partial^2 G^{\text{ex}}}{\partial x_c^2}\right) \quad (5)$$

it can be shown that the natural logarithm of the activity coefficient takes the form

$$\begin{aligned} \ln f_c = \beta [ & -2(A + 3B + 5C + 7D + 9E)x_c + \\ & (A + 9B + 29C + 61D + 105E)x_c^2 - \\ & (4B + 31C + 116D + 288E)x_c^3 + \\ & (12C + 20D + 408E)x_c^4 - (32D + 288E)x_c^5 + (80E)x_c^6 ] + \\ & F \frac{\sqrt{I}}{(1 + \sqrt{I})} + GI \end{aligned} \quad (6)$$

where the last two terms (first proposed by Guggenheim) have been added to ensure that the Debye–Hückel theory is satisfied for electrolytes at low salt concentrations.<sup>23,31</sup> Here,  $I$  is the ionic strength of the salt solution. The experimental data was fitted to the above form which provided an analytical expression for the required activity derivatives.

Errors in activity coefficients have the largest effect on the values obtained for the KB integrals.<sup>36</sup> In fact, errors in the fitting of activity coefficient derivatives from vapor pressure data can result in unphysical asymptotic values at either end of

**TABLE 1: Range of Compositions Investigated for the Cosolvent Systems<sup>a</sup>**

cosolvent	$\nu$	$\nu V^0$ (cm <sup>3</sup> /mol)	$10^5 \kappa_T$ (atm <sup>-1</sup> )	molar solubility limit	molar range of data used ( $x_c$ )
water	1	18.068	4.630		
NaCl	2	26.439	0.0	6.16	0.10–4.51 (0.0036–0.1526)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3	74.679	0.0	3.29, 5.76 <sup>b</sup>	0.10–2.41 (0.0054–0.1190)
GdmCl	2	69.996	0.0		0.93–6.96 (0.0348–0.3190)
urea	1	45.468	0.0	16.65	0.49–7.77 (0.0089–0.1776)
TFE	1	72.320	10.64	$\infty$	1.84–13.83 (0.0365–1.0000)

<sup>a</sup> Data correspond to  $T = 300$  K and  $p = 1$  atm. Symbols are  $\nu$ , the number of species in solution;  $\nu V^0 = V^0_{\text{salt}}$ , molar volume of pure component;  $\kappa_T$ , isothermal compressibility of the pure solution.  $V^0$  was determined from crystal structure data<sup>44–47</sup> for the salts and for urea.  $V^0$  for water and for TFE are from refs 89 and 39, respectively.  $\kappa_T$  was assumed to be zero for the pure crystals of the salts and urea.  $\kappa_T$  for water and TFE are from refs 89 and 49, respectively. Solubility limits are from reference 90, unless indicated otherwise. <sup>b</sup> From reference 91. TFE is miscible with water in all proportions.  $1 \text{ nm}^3 = 602 \text{ cm}^3/\text{mol}$ .

the composition range.<sup>36</sup> We did not observe this type of behavior during our analysis, although the data at low composition was subject to significant error. As a check of our data, we have also analyzed a more recent set of activity data for NaCl and urea solutions obtained from reference 37.

**Partial Molar Volumes.** Partial molar volumes for urea and TFE solutions were obtained directly from refs 38 and 39, respectively. The experimental densities for the NaCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, GdmCl, urea, and TFE solutions were obtained from refs 40, 41, 32, 42 and 39, respectively. For the NaCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions, the apparent molar volumes were then obtained from the densities using the relationship<sup>43</sup>

$$V_c^{\text{app}} = \frac{1000(\rho_w^0 - \rho)}{C_c \rho_w^0} + \frac{M_c}{\rho_w^0} \quad (7)$$

where  $\rho$  and  $\rho_w^0$  are the densities of the solution and pure water, respectively.  $M_c$  is the molar mass of the cosolvent, and  $C_c$  is the conventional molar concentration of the cosolvent. The apparent molar volume for the GdmCl solutions were obtained from reference 32. The partial molar volumes for these cosolvent solutions were then calculated using<sup>38</sup>

$$\bar{V}_{\text{salt}} = \nu \bar{V}_c = V_c^{\text{app}} + \left( \frac{1000 - C_c V_c^{\text{app}}}{1000 + C_c^2 \frac{\partial V_c^{\text{app}}}{\partial C_c}} \right) C_c \frac{\partial V_c^{\text{app}}}{\partial C_c} \quad (8)$$

where  $\bar{V}_{\text{salt}} = \nu \bar{V}_c$  since we assume that all the ions in the electrolyte are indistinguishable (see Appendix B). The values of the molar volume of the pure cosolvents are required for the reference state calculations. They were assumed to be equal to that of the salts in the solid state. Hence, they were obtained from crystal structure data for NaCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, GdmCl, and urea.<sup>44–47</sup> This is not the only possible choice, but it is quite reasonable in the absence of other data.

**Compressibilities.** Isothermal compressibilities for NaCl and urea solutions were taken from refs 40 and 48, respectively. For TFE/water mixtures, the adiabatic (rather than the isothermal) compressibility was obtained from ref 49. This is, however, not expected to contribute significantly to the error in the calculated KB integrals since the values of KB integrals are rather insensitive to the accuracy of the compressibility data used in the analysis. In fact, uncertainties as high as 50% in the compressibilities have been shown not to significantly affect the values of the KB integrals.<sup>36</sup> For ammonium sulfate and guanidinium chloride solutions, the compressibilities were not available as a function of composition. We have, therefore, assumed that the compressibility is a linear combination of the compressibilities of the pure components:

$$\kappa_T = \phi_w \kappa_T^w + \phi_c \kappa_T^c$$

**TABLE 2: Table of Fitted Constants (Eq 6)<sup>a</sup>**

cosolvent	A	B	C	D	E	F	G
NaCl	1.619	-0.263				-1.051	0.285
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.823	0.055				-2.354	0.124
GdmCl	-2.618	0.992				-0.959	-0.087
urea	-1.346	0.293	0.742				
TFE	3.448	1.674	0.391	0.482	0.183		

<sup>a</sup> Constants A–E are in units of (kJ/mol), while F and G are dimensionless.

where  $\kappa_T$ ,  $\kappa_T^w$ , and  $\kappa_T^c$  denote the compressibilities of the solution and of the pure components (water and cosolvent), respectively;  $\phi_w$  and  $\phi_c$  are the volume fractions of water and cosolvent in the solution. The values of the isothermal compressibility of the pure components are required in the calculation of KB integrals for the symmetric ideal reference state.  $\kappa_T^c$  was taken to be zero for all cosolvents except TFE.

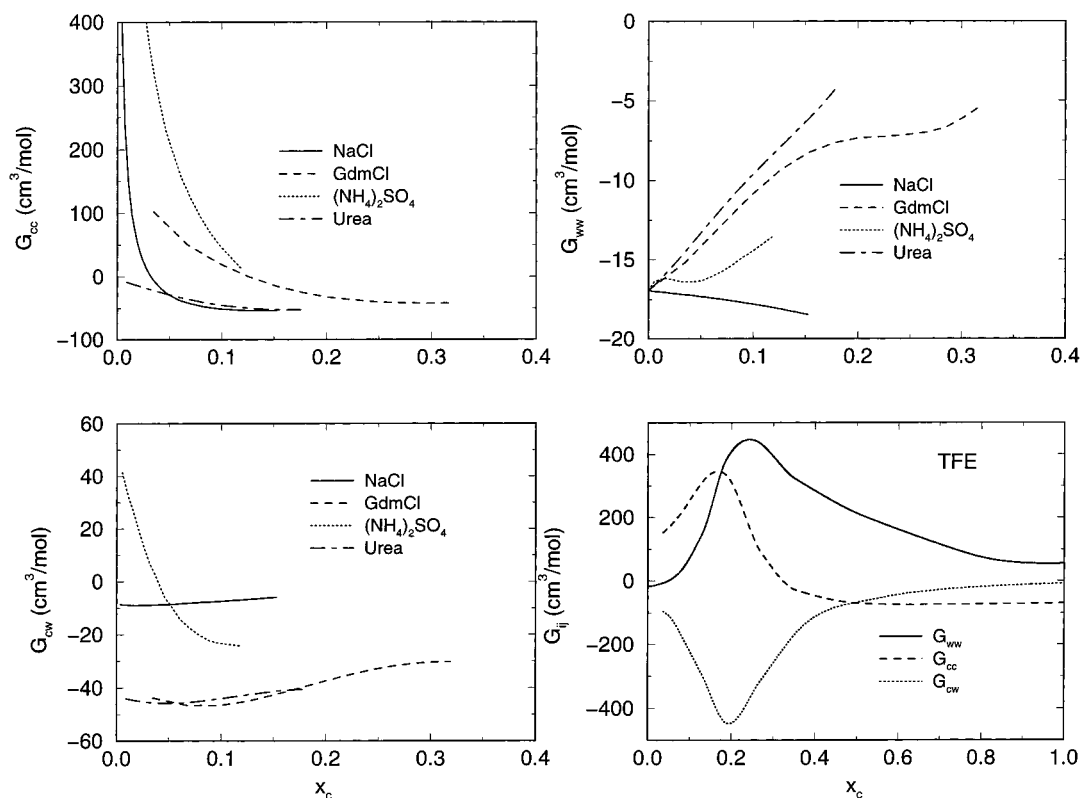
Table 1 lists the solubility limits of the cosolvents as well as the range of data used in this work to evaluate the KB integrals for these systems. Table 2 contains the fitted constants for eq 6. Urea solutions display the most ideal behavior, as has previously been noted.<sup>33</sup> The activity coefficients of TFE and water in TFE/water mixtures deviate significantly in comparison, as do the activity coefficients in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions. KB theory allows us to relate these differences to the relative distribution of species in solution.

Tables listing the partial molar volumes, compressibilities, activity coefficients, and their derivatives as a function of composition for all the cosolvent solutions studied here can be found in the Supporting Information.

## Results

The KB integrals for the four solutions have been determined using the appropriate experimental data (compressibility, activity coefficients and partial molar volumes) at 300 K and 1 atm, to give  $G_{\text{cc}}$ ,  $G_{\text{ww}}$ , and  $G_{\text{cw}}$ , as a function of composition. The results are presented in Figure 1. The experimental  $G_{\text{ww}}$  KB integrals for TFE, GdmCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and urea clearly display an increasing trend toward more positive values, while  $G_{\text{ww}}$  for NaCl displayed a small but opposite effect. In addition, the TFE changes were significantly larger than those for the other cosolvents and proceeded through a maximum at approximately  $x_c = 0.25$  (8 M or 57% v/v).

Our results at larger mole fractions of TFE are quantitatively different from a previous KB analysis of TFE solutions,<sup>50</sup> although the trend toward increasing  $G_{\text{ww}}$  at low mole fraction of alcohol ( $x_c < 0.25$ ) is consistent with the previous results. The exact reason for the quantitative disagreement is unclear at present. The data for NaCl is in good agreement with the results of Newman.<sup>51</sup> As expected, the  $G_{\text{cc}}$  integral diverges as



**Figure 1.** Experimentally determined Kirkwood–Buff integrals,  $G_{ww}$ ,  $G_{cc}$ , and  $G_{cw}$ , as a function of cosolvent mole fraction for NaCl, GdmCl,  $(\text{NH}_4)_2\text{SO}_4$ , urea, and TFE solutions.

the mole fraction of salt tends to zero.<sup>51,52</sup> Interestingly, the data also suggests a decrease in  $G_{cc}$  with increasing concentration for all cosolvents except TFE and maybe urea. The  $G_{cw}$  integral was relatively independent of composition for all but the  $(\text{NH}_4)_2\text{SO}_4$  and TFE solutions.  $G_{cw}$  increased significantly at low salt concentrations in the ammonium sulfate solutions, whereas  $G_{cw}$  first decreased and then increased with TFE mole fraction.

In Figure 2, the excess association numbers ( $\rho_j G_{ij}$ ) for one species around another are plotted as a function of composition. The excess self-association of urea molecules decreased on increasing the urea concentration. All the salts exhibited a maximum in cosolvent self-association at low cosolvent mole fraction. However, our analysis cannot accurately predict the position of this maximum due to the absence of experimental activity data at very low concentrations (see Table 1). Furthermore, if the salts obey Debye–Hückel theory at very low concentrations ( $10^{-3}$  M), the predicted values of  $\rho_c G_{cc}$  are larger than any of the corresponding maxima shown here. Hence, it appears that there could be more features, such as another maximum, in  $\rho_c G_{cc}$  at low salt concentrations which were not apparent here.

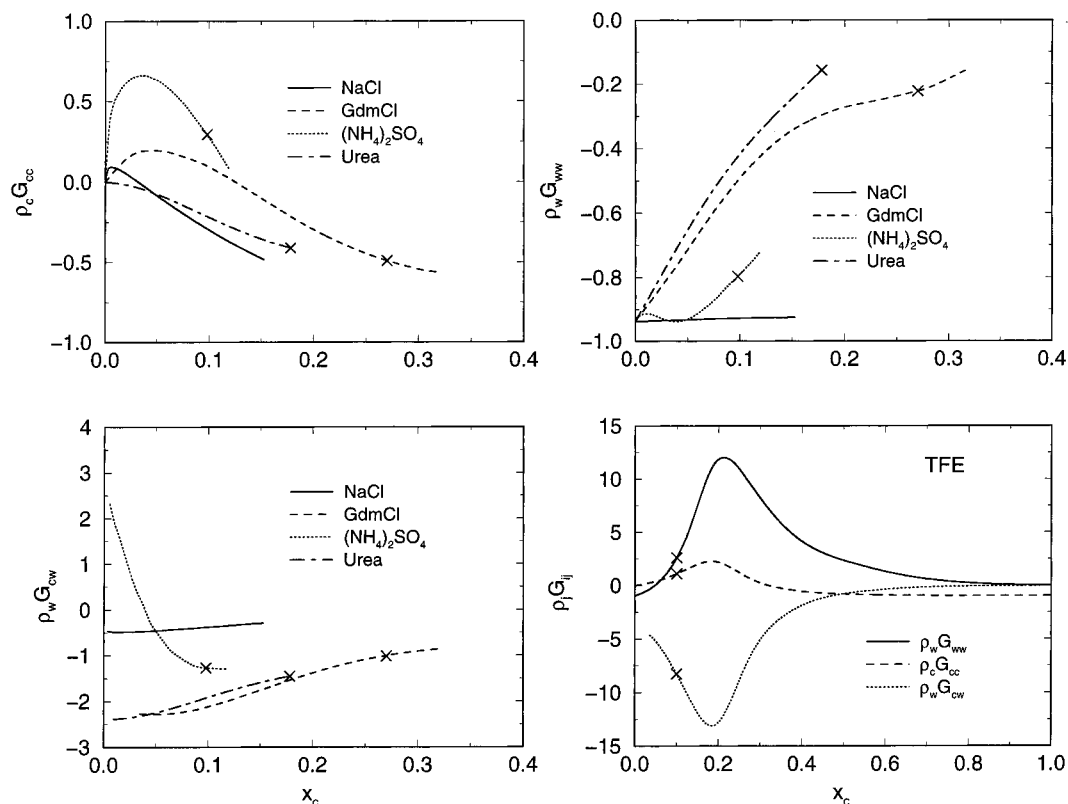
Self-association of ions was significantly higher in  $(\text{NH}_4)_2\text{SO}_4$  solutions. This is in agreement with recent theoretical and experimental studies.<sup>22,53</sup> There was a high degree of self-association ( $\rho_c G_{cc}$ ) as well as preferential hydration ( $\rho_w G_{cw}$ ) of the cosolvent in ammonium sulfate solutions up to 0.7M ( $x_c \sim 0.037$ ). Although this overcrowding contradicts physical intuition, the data is consistent with eq 11 (see Appendix A) and coincides with the range of concentrations for which the partial molar volume for ammonium sulfate is negative. The number of excess waters around water increased in all solutions except NaCl, for which it remained constant. Water self-association in TFE was large with a maximum at  $x_c = 0.209$  ( $\sim 7.27$  M or 51% v/v).

Excess hydration of the cosolvents ( $\rho_w G_{cw}$ ) indicated that ammonium sulfate was the only cosolvent which displayed an excess of water, and this only persisted for low concentrations. NaCl was slightly desolvated, while urea and GdmCl were significantly desolvated. Association of water with urea and GdmCl increased slightly with concentration. NaCl excess solvation displayed little variation in concentration, while  $(\text{NH}_4)_2\text{SO}_4$  molecules were substantially desolvated with increasing concentration.

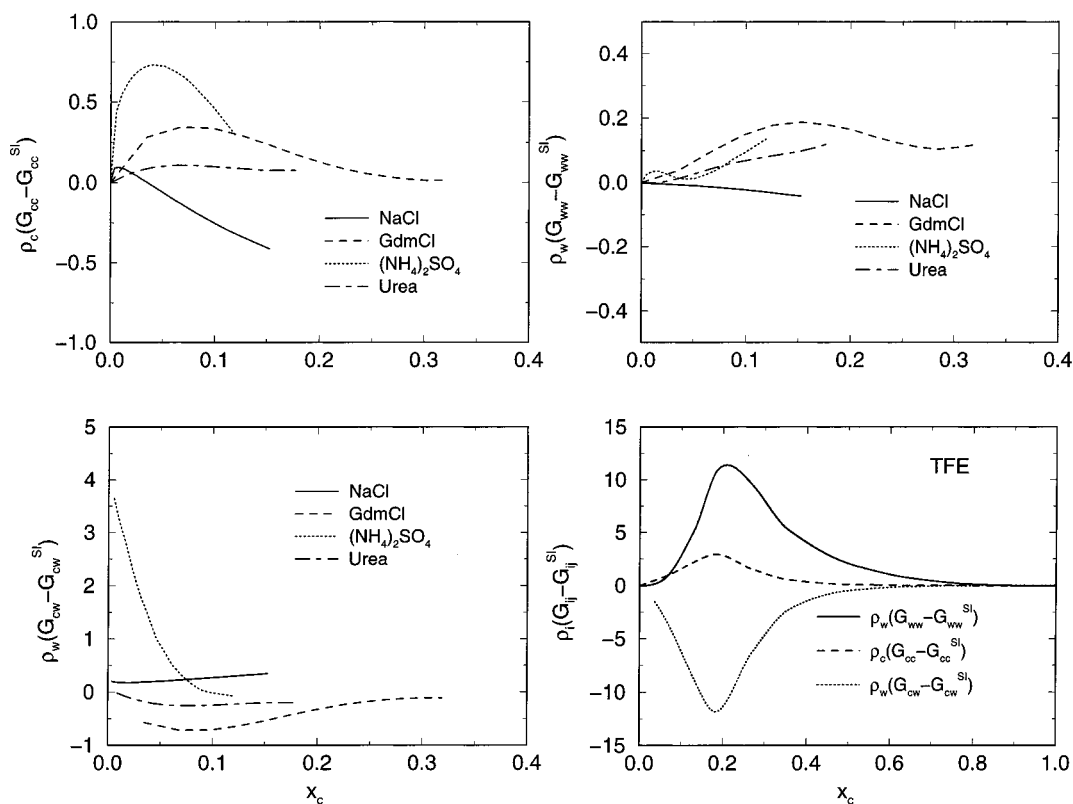
The above results describe the excess distribution (over random) of one species around another. However, one can observe changes in these distributions solely due to the packing of different size molecules and not due to specific attractive or repulsive interactions between them. This volume effect can be removed from the excess coordination numbers by using the symmetric ideal solution (not a random distribution) as a reference state (see Appendix C). The resulting data are displayed in Figure 3.

The values of  $\rho_w G_{ww}$  for TFE, being large in magnitude, were not appreciably affected by this correction, indicating that significant increases in the water intermolecular interactions occur on addition of TFE. On the other hand, the  $\rho_w G_{ww}$ ,  $\rho_c G_{cc}$ , and  $\rho_w G_{cw}$  associations obtained for urea were largely reproduced by simple volume changes. This agrees well with the often quoted observation of the ideal nature of urea solutions,<sup>54</sup> which display relatively small changes in activity with concentration of urea,<sup>33</sup> and is also in agreement with computer simulation results.<sup>8,55,56</sup> Furthermore, this also suggests that while the overall effect of urea on water structure is to increase water association, techniques which probe for changes in intermolecular interactions may detect very little change on introduction of urea as long as the results are normalized for the different urea concentrations involved. Using the symmetric ideal reference state, NaCl produced a slight decrease in the





**Figure 2.** Excess coordination numbers in solution as a function of composition for NaCl, GdmCl,  $(\text{NH}_4)_2\text{SO}_4$ , urea, and TFE solutions. The symbols indicate concentrations of 2, 6, 8, and 4.3 M (30% v/v TFE) for  $(\text{NH}_4)_2\text{SO}_4$ , GdmCl, urea, and TFE solutions, respectively.

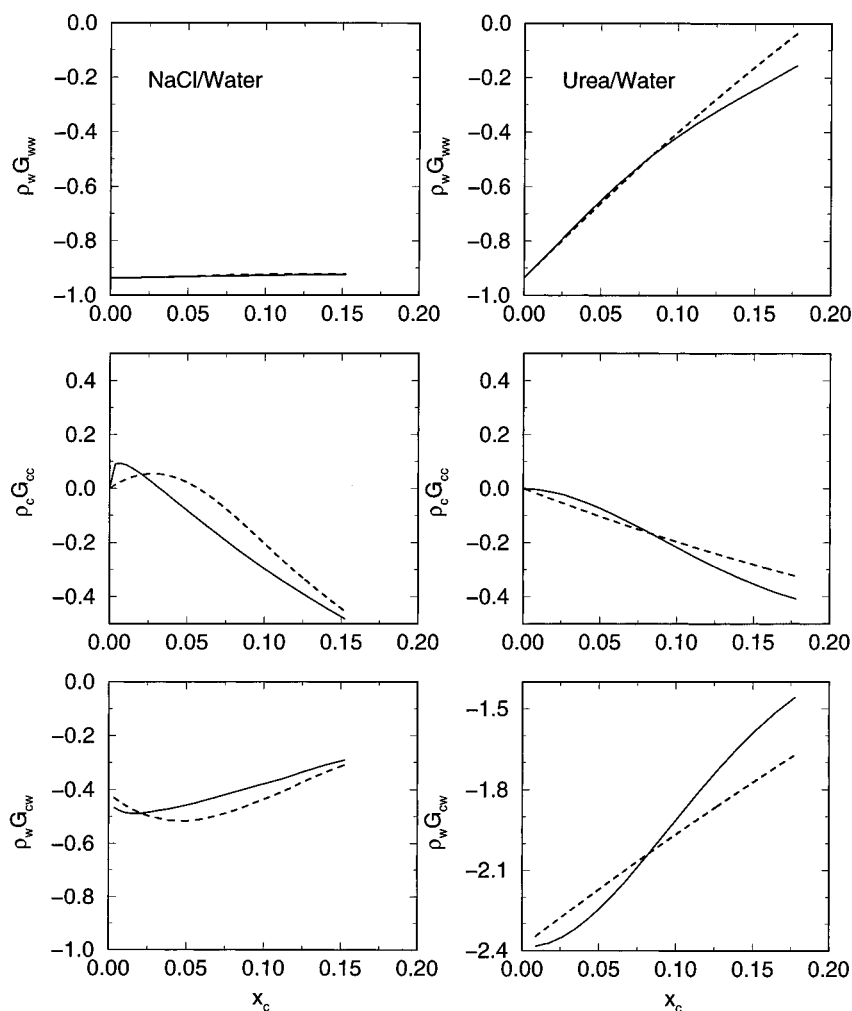


**Figure 3.** The differences between the excess association numbers for the real cosolvent solutions and the corresponding values for a symmetric ideal (SI) solution for different cosolvent compositions in NaCl, GdmCl,  $(\text{NH}_4)_2\text{SO}_4$ , urea, and TFE solutions.

interactions between water molecules, while GdmCl, urea, and  $(\text{NH}_4)_2\text{SO}_4$  all produced an increase in the water–water interactions. The data in Figures 3 and 4 emphasize that it is possible to observe different experimental trends depending on whether

the measurement is related to local changes in water density or changes in interactions (presumably hydrogen bonding).

The KB integrals are most sensitive to the activity derivatives used during the analysis.<sup>36</sup> Therefore, to provide a check of the



**Figure 4.** Excess association numbers determined using two different sets of activity data as a function of cosolvent mole fraction for NaCl and urea solutions. The solid lines are the data used here (reference 31) while the dotted lines represent data from ref 37. Panels on the left and right display data for NaCl and urea solutions, respectively.

results obtained here, we have repeated our NaCl and urea analysis using a different set of activity data.<sup>37</sup> The excess coordination numbers for both sets of data are displayed in Figure 4. While some quantitative differences were apparent, the trends described here were still evident in both sets of activity data. This provides a high degree of confidence in the present results.

## Discussion

It is gratifying to see that the trends in association of electrolyte solutions agree well with current descriptions of ion pairing and dissociation in salt solutions.<sup>57–59</sup> In particular, the initial increase in association and the subsequent decrease with increasing salt concentration was clearly observed in the present calculations (see  $\rho_c G_{cc}$  in Figure 2).

It is slightly surprising that the salt effects were typically smaller in magnitude in comparison with TFE. However, the KB integrals are integrals over rdfs which describe variations in the local composition of the solution over several molecular diameters. In this respect, it is possible that one can have regions of high or low association which then cancel to give no net excess coordination. Unfortunately, KB theory does not allow for a decomposition into distance dependent degrees of association. Here, one must use additional information such as that obtained from molecular simulations.<sup>60</sup>

It is tempting to associate increases in the distribution of water around water as an increase in water structure. However, this can be problematic. For instance, reasonable theories of cosolvent effects on the properties of water have been advanced which consider an equilibrium between higher density (bulk) and lower density (ice or clathrate) water structures.<sup>6</sup> A cosolvent which destroys the lower density (more structured) water might be considered to be a structure breaker and yet produce an increase in water density and therefore an increase in water–water coordination. The problem lies in the undefined, and maybe undefineable, concept of water structure. The present approach is based on the calculation of excess association numbers which can be directly related to the thermodynamics of solutions.<sup>26</sup> Using this information, we provide a discussion of excess association and water structure breaking/making arguments for each cosolvent.

**Sodium Chloride.** Addition of NaCl to water increases the surface tension and viscosity of the solution.<sup>2,18</sup> The solubility of alkanes in water is also reduced on the addition of NaCl (salting out effect). NaCl is considered to be a weak structure breaking salt on the basis of the observed variation of the viscosity changes with temperature (a better guide to structural changes than absolute viscosities),<sup>1</sup> which is supported by ultrasonic attenuation experiments.<sup>61</sup> However, differences in the entropy of bulk and coordination waters suggest both sodium and chloride ions are neither structure breakers or structure

makers.<sup>62</sup> This is somewhat surprising, as both sodium and chloride ions possess strong solvation shells.<sup>63</sup> The data presented here suggest that changes in excess water self-association and water–water interactions were relatively independent of concentration, with a possible slight structure breaking trend. This is in agreement with conductivity measurements of aqueous NaCl solutions where the authors inferred that the water structure remains unaffected over the full range of concentrations.<sup>64</sup> Association of sodium and chloride ions decreased with concentration after an initial small increase. The clustering of ions at low concentrations is in agreement with recent dynamic light scattering experiments of NaCl solutions,<sup>22</sup> as well as simulation studies on 1M NaCl.<sup>65,66</sup> The view of NaCl as a weak or indifferent water structure breaker is reasonable, even though the salting-out effect is more consistent with structure making. This conflict is removed if NaCl is assumed to increase the surface tension of water and salt out alkanes on the basis of the exclusion of salt ions from the vicinity of the interface or solute cavity, respectively, this effect more than compensating for any changes in water structure. Hence, the effects of NaCl on the solubility of hydrocarbons can be explained without invoking any significant water structure making or breaking propensity.

**Ammonium Sulfate.** Ammonium sulfate is known to increase the surface tension of water.<sup>2</sup> The temperature variation of the viscosity of ammonium sulfate solutions indicate that ammonium sulfate is a weak structure maker.<sup>18</sup> A study of the concentration dependence of the infrared librational band of water has indicated that ammonium sulfate produces a negative shift in the normalized first moment, suggesting that the number of hydrogen bonds are reduced in comparison to pure water. That is, the infrared studies suggest that ammonium sulfate is a structure breaker.<sup>67</sup> The former observation is in agreement with the data presented here (see Figures 2 and 3). However, inclusion of the  $(\rho^{SI}/\rho)$  term in eq 16 (see Appendix C) significantly reduces the estimated water–water interactions (Figure 3), which may help to explain the above experimental differences. The ability of ammonium sulfate to precipitate proteins can be rationalized by the increased surface tension and the subsequent exclusion of salt from the vicinity of the protein. Aggregation of  $(\text{NH}_4)_2\text{SO}_4$  in solution was significant and in agreement with previous experimental and simulation studies.<sup>22,53</sup> Also, the trend of decreasing solvation with increasing concentration in ammonium sulfate solutions is in agreement with previous calculations on secondary hydration numbers in salt solutions.<sup>68</sup>

**Guanidinium Chloride.** Solutions of GdmCl also display increased surface tension and viscosity.<sup>42,69</sup> The increase in surface tension is expected due to the probable exclusion of the ions from an interface. The results of the present KB analysis suggest that GdmCl increases absolute water association over pure water and that water–water interactions are also slightly increased. Both these observations indicate a tendency for structure making. In contrast to NaCl, GdmCl solutions exhibit a salting in effect on alkanes, with the exception of methane which is salted out.<sup>13</sup> Our previous computational studies of salting in/out effects reproduced the salting out effect of guanidinium chloride on methane.<sup>53</sup> A trend observed in those studies was a tendency for increased association of  $\text{Gdm}^+$  ions with larger hydrophobic groups at high GdmCl concentrations. If this trend continued, association of  $\text{Gdm}^+$  ions with larger alkanes could explain the salting in effects of GdmCl solutions on these solutes.

**Urea.** Urea has been studied extensively by a variety of experimental techniques. Urea increases the surface tension and viscosity of water solutions,<sup>42</sup> and changes in the temperature coefficients of the viscosity suggest that urea is a weak structure maker<sup>1,70</sup> (more recent viscosity results have been inconclusive<sup>18</sup>). A recent NMR study of the diffusivity of water in urea solutions indicates that urea does not affect the structure of water to a great extent and might possibly be classified as a mild structure maker.<sup>71</sup> Neutron diffraction studies and internal pressure measurements provided no evidence for either structure making or breaking.<sup>72,73</sup> However, an early Raman spectroscopic study indicated urea was a structure breaker,<sup>11</sup> and this was supported by ultrasonic attenuation measurements.<sup>70</sup> As mentioned earlier, the most cited evidence for the structure breaking effects of urea is the increase in solubility of alkanes (except methane and ethane) with urea concentration,<sup>13</sup> as explained by a simple model developed to interpret those data based on changes in water structure.<sup>6</sup> Unfortunately, the above model did not consider the possibility of urea association with the hydrocarbon. This had been ruled out on the basis of the positive enthalpies of transfer from water to urea solutions.<sup>6</sup> However, a positive enthalpy does not necessarily exclude urea binding to nonpolar solutes. In fact, urea binding could result in a positive enthalpy change (slight desolvation of urea), compensated by a favorable entropy change arising from the release of bound water.

Our previous computational studies of the salting out effect of urea on methane<sup>53</sup> are in good agreement with the experimental data<sup>74</sup> and suggest that association of urea with a nonpolar solute increases with increasing solute size. Hence, it seems logical to conclude that the increase in alkane solubility arises from an excess of ureas in the vicinity of the alkane which is sufficient to salt in alkanes larger than ethane. The effect on methane solubility, where the overall binding appears to be low, is consistent with an increase in water structure and/or an exclusion of urea from the solute. A slight increase in water structure is also supported by recent NMR<sup>14</sup> and computer simulation results<sup>8,9</sup> and by the KB analysis of the experimental thermodynamic data presented here. In conclusion, the designation of urea as a slight water structure maker does not conflict with the majority of the available experimental data.

**TFE.** Solutions of TFE possess very interesting properties. At low mole fractions of TFE, they are commonly observed to increase protein or peptide structure and to promote the formation of  $\alpha$ -helices and  $\beta$ -sheets. At higher mole fractions, TFE solutions behave similarly to most alcohols and denature proteins. This change in behavior is also found in viscosity measurements of TFE–water mixtures where the viscosity proceeds through a maximum at  $x_c \sim 0.2$  (7 M or 50% v/v) of TFE,<sup>75</sup> in agreement with the current KB analysis (see Figure 3). In addition, NMR studies have determined that clustering of TFE molecules is also dependent on concentration, with a maximum around  $x_c = 0.1$  (4.3 M or 30% v/v) of TFE.<sup>76</sup> TFE is assumed to be a structure maker,<sup>77</sup> and this effect was supported by our KB analysis of the experimental thermodynamic data. Our previous simulation results on methane solubility in TFE solutions<sup>53</sup> also indicate a large salting in effect supporting the view of TFE interacting strongly with hydrophobic groups.<sup>78</sup> The surface tension of water is decreased on introduction of TFE,<sup>79,80</sup> suggesting that an excess of TFE is present at the surface. Hence, TFE is a structure maker but displays a strong salting in effect. Clearly, these two effects will tend to stabilize and destabilize protein structure, respec-

tively. It is possible that low concentrations of TFE favor the former (stabilizing) effect, while more concentrated solutions will favor the latter (destabilizing) effect. However, the exact mechanism for TFE effects on peptides and proteins still remains unclear.<sup>81</sup>

### Conclusions

An understanding of the properties of cosolvent solutions is required before one can fully rationalize the effects of cosolvents on the structure and stability of biomolecules. We have used Kirkwood–Buff theory to analyze the available experimental data to investigate the changes in associations and interactions on addition of some common cosolvents. Mixtures of TFE and water produced large changes in the properties of the solution. Somewhat surprisingly the addition of ionic species displayed far smaller overall effects. The KB results provide information on molecular associations in solution which can be used to rationalize a variety of experimental data. While effects on water association are obviously important for determining the properties of cosolvent solutions, using water structure making or breaking arguments to explain the effects of cosolvents on protein stabilization and solubility appears to be too simple and should be avoided in all but a few cases.<sup>82</sup> In most instances, the binding or exclusion of the cosolvent will also contribute and these facts highlight why there is no correlation between the effects of cosolvents on proteins and their effects on the activity of water.<sup>1</sup>

### Appendix A: Relationships between the KB integrals

From the physical consideration that the volume not occupied by one species must be occupied by the other, it has been proposed that the following relationship holds for binary mixtures:<sup>83</sup>

$$\rho_i \bar{V}_i G_{ii} + \rho_j \bar{V}_j G_{ij} = N_{ii} \bar{V}_i + N_{ij} \bar{V}_j = 0 \quad (9)$$

However, expressing the partial molar volumes in terms of KB integrals,<sup>26</sup> one finds that the KB integrals actually satisfy the following relationship:

$$\rho_i \bar{V}_i G_{ii} + \rho_j \bar{V}_j G_{ij} = RT\kappa_T - \bar{V}_i \quad (10)$$

for any composition of the cosolvent solution. The term  $\bar{V}_i$  arises due to the finite volume excluded by the molecule under consideration, while the term involving the compressibility is derived from the fact that the KB integrals are defined in the grand canonical ensemble. This type of relationship is also observed for single component solutions and was derived by Ben-Naim for the case of an infinitely dilute solute in a binary solution.<sup>26</sup> We postulate that the following relation should hold at all compositions for a solution with  $n$  components:

$$\sum_{j=1}^n \rho_j \bar{V}_j G_{ij} = RT\kappa_T - \bar{V}_i \quad (11)$$

All the KB integrals presented here obey the above relationship to within 0.01 cm<sup>3</sup>/mol.

### Appendix B: Electroneutrality

Complications arise when KB theory is applied to electrolyte solutions. Due to the restriction of electroneutrality, KB theory cannot be applied directly to generate equations for a ternary solution of solvent, cations, and anions ( $w$ ,  $+$ ,  $-$ ). Hence, one cannot obtain individual cation or anion activity derivatives or

partial molar volumes. To avoid this problem, we have assumed that the cations and anions are indistinguishable and used the corresponding equations for a binary solution. This approach has been adopted before to avoid problems associated with electroneutrality.<sup>84</sup>

Since the cations and anions are assumed to be indistinguishable, the following definition for the mole fraction of cosolvent in solution has been adopted:

$$x_c = \frac{\nu m_c}{\nu m_c + m_w} \quad (12)$$

where  $m_c$  and  $m_w$  are the molalities of cosolvent and water, respectively, and  $\nu$  is the number of ions obtained on dissociation of one molecule of the electrolyte. This is different from the conventional definition, which is given by eq 12 without the  $\nu$  in the numerator.<sup>31</sup> All figures, tables and data refer to the above definition (eq 12) unless stated otherwise.

Consequently, for a salt of the form  $M_{\nu^+}X_{\nu^-}$ , we have  $\nu = \nu^+ + \nu^-$ ;  $\rho_c = \rho_+ + \rho_- = \nu \rho_{\text{salt}}$ ;  $\mu_c = (1/\nu)(\nu^+ \mu_+ + \nu^- \mu_-)$ ;  $\bar{V}_c = (1/\nu)(\nu^+ \bar{V}_+ + \nu^- \bar{V}_-)$ ;  $\gamma_c^\nu = \gamma_{\pm}^\nu = \gamma_+^{\nu^+} \gamma_-^{\nu^-}$ ;  $f_c^\nu = f_{\pm}^\nu = f_+^{\nu^+} f_-^{\nu^-}$ . Also, if  $z^+$  and  $z^-$  are the charges on the cation and anion, respectively, the conditions for electroneutrality are

$$\begin{aligned} z^+ + z^- \rho_+ G_{++} + z^- \rho_- G_{+-} &= 0 \\ z^- + z^- \rho_- G_{--} + z^+ \rho_+ G_{+-} &= 0 \\ z^+ \rho_+ G_{+w} + z^- \rho_- G_{-w} &= 0 \end{aligned} \quad (13)$$

The above equations can be used to relate  $G_{cc}$  and  $G_{cw}$  to the individual KB integrals between the various species in solution:

$$\begin{aligned} \rho_c G_{cc} &= \rho_+ G_{++} + \rho_- G_{+-} = \rho_c G_{+-} - 1 \\ \rho_c G_{cw} &= \rho_+ G_{+w} + \rho_- G_{-w} = \rho_c G_{+w} \end{aligned} \quad (14)$$

Using the above relations, the expressions for thermodynamic quantities derived using the KB integrals with the assumption of indistinguishability are found to be identical to expressions obtained by Kusalik and Patey for ionic solutions after defining  $k$ -dependent KB integrals and taking the  $k \rightarrow 0$  limit analytically,<sup>52</sup> and also with the expressions derived using the method of generalized inverses.<sup>85</sup> Hence, consistent expressions can be obtained for the KB integrals as long as the anions and cations are not treated as distinct species in solution. Furthermore, the results obtained here with the assumption of indistinguishability of ions are in agreement with the results derived by Newman from a KB analysis of salt solutions using a different approach.<sup>51,86</sup> Using eq 14, one can obtain  $G_{+-}$  from  $G_{cc}$  and therefore,  $G_{++}$  and  $G_{--}$  as a function of concentration.

Using Debye–Hückel theory, it can also be shown that at infinite dilution of a 1:1 salt,  $G_{+-} = \infty$ ,  $\rho_+ G_{+-} = 0.5$ ,  $\rho_+ G_{++} = \rho_- G_{--} = -0.5$ ,<sup>51,52</sup> which implies that  $G_{cc} \rightarrow \infty$  and  $\rho_c G_{cc} \rightarrow 0$ . In general, for a salt of the form  $M_{\nu^+}X_{\nu^-}$ , we have the following relations at infinite dilution of salt:  $\rho_+ G_{+-} = \nu^+/\nu$ ,  $\rho_- G_{+-} = \nu^-/\nu$ ,  $\rho_+ G_{++} = -\nu^-/\nu$ ,  $\rho_- G_{--} = -\nu^+/\nu$ , which again leads to the condition  $\rho_c G_{cc} \rightarrow 0$ . At very low salt concentrations ( $10^{-3}$  M), the values of  $\rho_c G_{cc}$  are predicted to be 0.33 (1:1 salt) and 0.94 (1:2 salt) using Debye–Hückel theory.

### Appendix C: Reference States

The KB theory of solutions provides an estimate of the excess or deficit of one species around another as a consequence of



the preferential interactions between them. However, preferential interactions can arise due to both favorable interactions and/or due to the different sizes of the species involved. Recently, Matteoli has used KB theory to decompose the KB integrals into two components, one originating from differences between the relative sizes (excluded volume) of the cosolvent and solvent molecules, and the other from changes in the intermolecular interactions.<sup>27</sup> Here, to estimate the role of molecular interactions, it is necessary to examine the difference between the KB integrals of the given system with those of a corresponding symmetric ideal (SI) solution, where the activity coefficient is unity and the partial molar volume ( $\bar{V}^\circ$ ) of each species is constant and equal to the value for the pure components.<sup>27</sup> The compressibility of a symmetric ideal solution is taken to be a linear function of the volume fractions of the components:

$$\kappa_T = \phi_w^\circ \kappa_T^w + \phi_c^\circ \kappa_T^c$$

where

$$\phi_i^\circ = \frac{x_i \bar{V}_i^\circ}{x_w \bar{V}_w^\circ + x_c \bar{V}_c^\circ}$$

is the volume fraction of the  $i$ th component, and  $\kappa_T^w$  and  $\kappa_T^c$  are the compressibilities of water and pure cosolvent, respectively. The expressions for the KB integrals for a symmetric ideal mixture are then given by<sup>27</sup>

$$\begin{aligned} G_{cw}^{SI} &= RT\kappa_T^c - \bar{V}_w^\circ - \phi_w^\circ(\bar{V}_c^\circ - \bar{V}_w^\circ + RT\kappa_T^c - RT\kappa_T^w) \\ G_{ww}^{SI} &= G_{cw}^{SI} + \bar{V}_c^\circ - \bar{V}_w^\circ; \quad G_{cc}^{SI} = G_{cw}^{SI} - \bar{V}_c^\circ + \bar{V}_w^\circ \end{aligned} \quad (15)$$

The KB integrals are found to be nonzero in this case. If the partial molar volume of the cosolvent is larger than for water, then  $G_{ww}^{SI}$  will naturally increase on addition of cosolvent even though the activity remains at unity, and therefore, the intermolecular interactions are unaffected. Hence, one can decouple simple relative volume effects from changes in the interactions between species. However, a symmetric ideal solution of ions will not obey the electroneutrality conditions since those relations arise as a consequence of interactions between ions, which are absent in a symmetric ideal solution.

Other reference states have been suggested.<sup>87,88</sup> These have primarily arisen due to the fact that the symmetric ideal KB integrals do not obey eq 9 (see Appendix A). As mentioned previously, this relationship is only an approximation, and one finds that the KB integrals for the SI solution do obey the correct relationship (eq 10) (see Appendix A). Even so, we have also investigated the reference state proposed by Ruckenstein et al.<sup>87,88</sup> with no change in the observed trends for the cosolvent solutions studied here.

To estimate changes in association due to intermolecular interactions, over and above that due to size effects, one has to subtract the corresponding value for a symmetric ideal solution

$$\Delta n_{ij} = \rho_j G_{ij} - \rho_j^{SI} G_{ij}^{SI} = \rho_j \left( G_{ij} - \frac{\rho_j^{SI} G_{ij}^{SI}}{\rho_j} \right) \quad (16)$$

Typically, the ratio ( $\rho_j^{SI}/\rho_j$ ) is assumed to be 1 in the literature.<sup>83,88</sup> This assumption involves an error of about 3% for most solutions, but is significant for (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions (~13%). However, only  $\Delta n_{ww}$  in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions is appreciably

affected by this approximation and we have therefore taken the more common approach of assuming ( $\rho_j^{SI}/\rho_j$ ) = 1 during our analysis.

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**Supporting Information Available:** Tables listing experimental values for partial molar volumes, activity coefficient derivatives (raw and fitted), compressibilities and the values of the Kirkwood–Buff integrals calculated using these data for NaCl, ammonium sulfate, GdmCl, urea, and TFE solutions. The values for  $G_{ij}$  for symmetric ideal binary mixtures of the cosolvent solutions studied here are also listed as a function of mole fraction in a separate table. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- (1) von Hippel, P. H.; Schleich, T. The Effects of Neutral Salts on the Structure and Conformational Stability of Macromolecules in Solution. In *Biological Macromolecules*; Timasheff, S. N., Fasman, G. D., Eds.; Marcel Dekker: New York, 1969; Vol. 2, pp 417–574.
- (2) Collins, K. D.; Washabaugh, M. L. *Q. Rev. Biophys.* **1985**, *18*, 323–422.
- (3) Timasheff, S. N.; Arakawa, T. Stabilization of Protein Structure by Solvents. In *Protein Structure: A Practical Approach*; Creighton, T. E., Ed.; IRL Press: London, 1989; pp 331–345.
- (4) Rupley, J. A. *J. Phys. Chem.* **1964**, *68*, 2002–2003.
- (5) Abu-Hamdiyyah, M. *J. Phys. Chem.* **1965**, *69*, 2720–2725.
- (6) Frank, H. S.; Franks, F. *J. Chem. Phys.* **1968**, *48*, 4746–4757.
- (7) Barone, G.; Rizzo, E.; Vitagliano, V. *J. Phys. Chem.* **1970**, *74*, 2230–2232.
- (8) Wallqvist, A.; Covell, D. G.; Thirumalai, D. *J. Am. Chem. Soc.* **1998**, *120*, 427–428.
- (9) Vanzi, F.; Madan, B.; Sharp, K. *J. Am. Chem. Soc.* **1998**, *120*, 10748–10753.
- (10) Franks, F.; Eagland, D. C. R. *Crit. Rev. Biochem.* **1975**, *3*, 165–219.
- (11) Walrafen, G. E. *J. Chem. Phys.* **1966**, *44*, 3726–3727.
- (12) Tsangaris, J. M.; Martin, R. B. *Arch. Biochem. Biophys.* **1965**, *112*, 267–272.
- (13) Wetlaufer, D. B.; Malik, S. K.; Stoller, L.; Coffin, R. L. *J. Am. Chem. Soc.* **1964**, *86*, 508–514.
- (14) Yoshida, K.; Ibuki, K.; Ueno, M. *J. Chem. Phys.* **1998**, *108*, 1360–1367.
- (15) Lamanna, R.; Cannistraro, S. *Chem. Phys.* **1996**, *213*, 95–110.
- (16) Tongraar, A.; Liedl, K. R.; Rode, B. M. *J. Phys. Chem. A* **1998**, *102*, 10340–10347.
- (17) Harris, K. R.; Newitt, P. J. *J. Phys. Chem. B* **1998**, *102*, 8874–8879.
- (18) Banerjee, R.; Frilley, B.; Guissani, A. *Ind. J. Biochem. Biophys.* **1999**, *36*, 107–117.
- (19) Ren, X.; Lin, R.; Ni, Y.; Zong, H. *Can. J. Chem.* **1999**, *77*, 387–390.
- (20) Harris, K. R.; Newitt, P. J.; Derlacki, Z. J. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 1963–1970.
- (21) Kuprin, S.; Gräslund, A.; Ehrenberg, A.; Koch, M. H. J. *Biochem. Biophys. Res. Comm.* **1995**, *217*, 1151–1156.
- (22) Georgalis, Y.; Kierzek, A. M.; Saenger, W. *J. Phys. Chem. B* **2000**, *104*, 3405–3406.
- (23) Guggenheim, E. A. *Philos. Mag.* **1935**, *19*, 588–643.
- (24) Pitzer, K. S. *J. Phys. Chem.* **1973**, *77*, 268–277.
- (25) Kirkwood, J. G.; Buff, F. P. *J. Chem. Phys.* **1951**, *19*, 774–777.
- (26) Ben-Naim, A. *Statistical Thermodynamics for Chemists and Biochemists*; Plenum Press: New York, 1992.
- (27) Matteoli, E. *J. Phys. Chem. B* **1997**, *101*, 9800–9810.
- (28) Ben-Naim, A. *J. Chem. Phys.* **1977**, *67*, 4884–4890.

- (29) Ben-Naim, A. Inversion of Kirkwood-Buff Theory of Solutions and Its Applications. In *Advances in Thermodynamics. Fluctuation Theory of Mixtures*; Matteoli, E., Mansoori, G. A., Eds.; Taylor & Francis: New York, 1990; Vol. 2, pp 211–226.
- (30) Weerasinghe, S.; Pettitt, B. M. *Mol. Phys.* **1994**, *82*, 897–912.
- (31) Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions*; Butterworths: London, 1959.
- (32) Makhatadze, G. I.; Fernandez, J.; Freire, E.; Lilley, T. H.; Privalov, P. L. *J. Chem. Eng. Data* **1993**, *38*, 83–87.
- (33) Stokes, R. H. *Aust. J. Chem.* **1967**, *20*, 2087–2100.
- (34) Cooney, A.; Morcom, K. W. *J. Chem. Thermodyn.* **1988**, *20*, 735–741.
- (35) Redlich, O.; Kister, A. T. *J. Am. Chem. Soc.* **1949**, *71*, 505.
- (36) Matteoli, E.; Lepori, L. *J. Chem. Phys.* **1984**, *60*, 2856–2863.
- (37) Miyawaki, O.; Saito, A.; Matsuo, T.; Nakamura, K. *Biosci. Biotech. Biochem.* **1997**, *61*, 466–469.
- (38) Gucker, F. T., Jr.; Gage, F. W.; Moser, C. E. *J. Am. Chem. Soc.* **1938**, *60*, 2582–2588.
- (39) Rochester, C. H.; Symonds, J. R. *J. Fluorine Chem.* **1974**, *4*, 141–148.
- (40) Rogers, P. S. Z.; Pitzer, K. S. *J. Phys. Chem. Ref. Data* **1982**, *11*, 15–81.
- (41) Perry, R. H.; Green, D. W. *Perry's Chemical Engineers' Handbook*, 7th ed.; McGraw-Hill: New York, 1997.
- (42) Kawahara, K.; Tanford, C. J. *Biol. Chem.* **1966**, *241*, 3228–3232.
- (43) Gucker, F. T. *J. Chem. Rev.* **1933**, *13*, 111–130.
- (44) Levine, I. N. *Physical Chemistry*, 4th ed.; McGraw-Hill: New York, 1995.
- (45) Ahmed, S.; Shamah, A. M.; Kamel, R.; Badr, Y. *Phys. Stat. Solidi A* **1987**, *99*, 131–140.
- (46) Haas, D. J.; Harris, D. R.; Mills, H. H. *Acta Cryst.* **1965**, *19*, 676–679.
- (47) Worsham, J. E., Jr.; Levy, H. A.; Peterson, S. W. *Acta Cryst.* **1957**, *10*, 319–323.
- (48) Endo, H. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 1106–1111.
- (49) Patil, K. J.; Ali, S. I. *Ind. J. Pure App. Phys.* **1981**, *19*, 617–619.
- (50) Blandamer, M. J.; Burgess, J.; Cooney, A.; Cowles, H. J.; Horne, I. M.; Martin, K. J.; Morcom, K. W.; Warrick, P., Jr. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 2209–2213.
- (51) Newman, K. E. *Chem. Soc. Rev.* **1994**, *23*, 31–40.
- (52) Kusalik, P. G.; Patey, G. N. *J. Chem. Phys.* **1987**, *86*, 5110–5116.
- (53) Smith, P. E. *J. Phys. Chem. B* **1999**, *103*, 525–534.
- (54) Franks, F. Effect of Solutes on the Hydrogen Bonding in Water. In *Hydrogen-bonded Solvent Systems*; Covington, A. K., Jones, P., Eds.; Taylor & Francis: London, 1968.
- (55) Tanaka, H.; Nakanishi, K.; Touhara, H. *J. Chem. Phys.* **1985**, *82*, 5184–5191.
- (56) Hernandez-Cobos, J.; Ortega-Blake, I.; Bonilla-Marin, M.; Moreno-Bello, M. *J. Chem. Phys.* **1993**, *99*, 9122–9134.
- (57) Davies, C. W. *Ion Association*; Butterworths: London, 1962.
- (58) Pitzer, K. S. Ion Interaction Approach: Theory and Data Correlation. In *Activity Coefficients in Electrolyte Solutions*, 2nd ed.; Pitzer, K. S., Ed.; CRC Press: New York, 1991.
- (59) Felmy, A. R.; Weare, J. H. *SSSA Spec. Publ.* **1995**, *42*, 31–52.
- (60) Chitra, R.; Smith, P. E. *J. Chem. Phys.* **2001**, *114*, 426–435.
- (61) Hammes, G. G.; Swann, J. C. *Biochemistry* **1967**, *6*, 1591–1596.
- (62) Samoilov, O. Y. Residence Times of Ionic Hydration. In *Water and Aqueous Solutions*; Horne, R. A., Ed.; John Wiley & Sons: New York, 1972.
- (63) Chitra, R.; Smith, P. E. *J. Phys. Chem. B* **2000**, *104*, 5854–5864.
- (64) Roberts, J. A.; Zhang, X.; Zheng, Y. *J. Chem. Phys.* **1994**, *100*, 1503–1510.
- (65) Degreve, L.; da Silva, F. L. B. *J. Chem. Phys.* **1999**, *110*, 3070–3078.
- (66) Degreve, L.; da Silva, F. L. B. *J. Chem. Phys.* **1999**, *111*, 5150–5156.
- (67) James, D. W.; Frost, R. L. *J. Chem. Soc., Faraday Trans.* **1977**, 583–596.
- (68) Luck, W. A. P. *4th Int. Symp. Fresh Water Sea* **1973**, *4*, 531–538.
- (69) Breslow, R.; Guo, T. *Proc. Natl. Acad. Sci., USA* **1990**, *87*, 167–169.
- (70) Hammes, G. G.; Schimmel, P. R. *J. Am. Chem. Soc.* **1967**, *89*, 442–446.
- (71) Mayele, M.; Holz, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2429–2434.
- (72) Finney, J. L.; Soper, A. K.; Turner, J. *Phys. B* **1989**, *156 & 157*, 151–153.
- (73) Dack, M. R. *J. Aust. J. Chem.* **1976**, *29*, 771–778.
- (74) Clever, H. L.; Young, C. L. *IUPAC Solubility Data Series. Volume 27/28. Methane*; Pergamon Press: Oxford, 1987.
- (75) Murto, J.; Heino, E. L. *Suomen Kemistilehti B* **1966**, *39*, 263–266.
- (76) Hong, D. P.; Hoshino, M.; Kuboi, R.; Goto, Y. *J. Am. Chem. Soc.* **1999**, *121*, 8427–8433.
- (77) MacDonald, D. D.; Dolan, B.; Hyne, J. B. *J. Solution Chem.* **1976**, *5*, 405–416.
- (78) Rajan, R.; Awasthi, S. K.; Bhattacharjya, S.; Balaram, P. *Biopolymers* **1997**, *42*, 125–128.
- (79) Paluch, M.; Dynarowicz, P. *J. Colloid Interface Sci.* **1984**, *98*, 131–137.
- (80) Gente, G.; La Mesa, C. *J. Solution Chem.* **2000**, *29*, 1159–1172.
- (81) Buck, M. *Q. Rev. Biophys.* **1998**, *31*, 297–355.
- (82) Kuharski, R. A.; Rossky, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 5786–5793.
- (83) Matteoli, E.; Lepori, L. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 431–436.
- (84) Friedman, H. L.; Ramanathan, P. S. *J. Phys. Chem.* **1970**, *74*, 3756–3765.
- (85) Behera, R. *J. Chem. Phys.* **1998**, *108*, 3373–3374.
- (86) Newman, K. E. Application of Kirkwood-Buff Theory to Electrolyte Solutions. In *Advances in Thermodynamics. Fluctuation Theory of Mixtures*; Matteoli, E., Mansoori, G. A., Eds.; Taylor & Francis: New York, 1990; Vol. 2, pp 373–398.
- (87) Shulgin, I.; Ruckenstein, E. *J. Phys. Chem. B* **1999**, *103*, 4900–4905.
- (88) Ruckenstein, E.; Shulgin, I. *J. Phys. Chem. B* **1999**, *103*, 10266–10271.
- (89) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. *J. Phys. Chem.* **1987**, *91*, 6269–6271.
- (90) Dean, J. A. *Lange's Handbook of Chemistry*; McGraw-Hill: New York, 1992.
- (91) Mohan, R.; Kaytancioglu, O.; Myerson, A. S. *J. Cryst. Growth* **2000**, *217*, 393–403.