Biophysical Chemistry 251 (2019) 106175

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Quantitative interpretation of isopiestic measurements on aqueous solutions: Urea revisited

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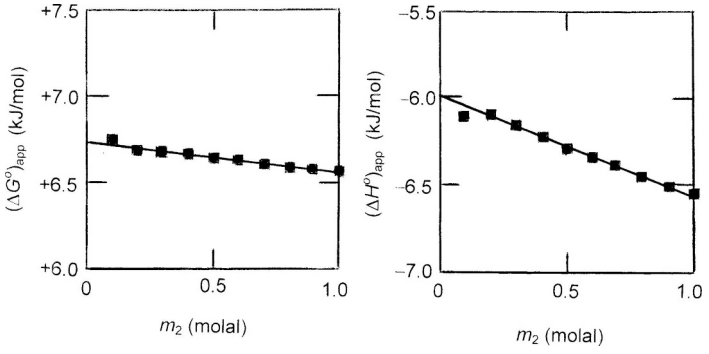
HIGHLIGHTS

- Expressions for rigorous thermodynamic analysis of isopiestic measurements
- Upward revision of the dimerization constant to 0.066 molal^{-1} for urea in aqueous solution at 25 °C
- Identification of the error in an earlier estimate of 0.033 molal^{-1} from the same isopiestic data
- Over-compensation by the standard entropy term that leads to a positive standard free energy change despite a sizeable negative enthalpic contribution
- Correlation of the large negative entropic contribution to the energetics of urea dimerization with a quantum-mechanics-based chemical structure showing the adverse effects of changes in water structure on dimer formation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Enthalpy-entropy compensation
Isopiestic measurements
Solvent interactions
Thermodynamic nonideality
Urea dimerization

ABSTRACT

This investigation amends the analysis of isopiestic measurements of solvent thermodynamic activity by taking into account the fact that the solvent activity, traditionally expressed in mole-fraction terms, is a molal parameter because of the constraints (constant temperature and pressure) under which the measurements are made. Application of the revised procedure to published isopiestic measurements on aqueous urea solutions at 25 °C yields a dimerization constant of 0.066 molal^{-1}, which is two-fold larger than an earlier published estimate based on an incorrect definition of the solute activity coefficient. Despite amendments to the quantitative detail, the present study confirms the existence of a large negative entropic contribution that largely counters its enthalpic counterpart arising from the hydrogen bonding responsible for dimer formation. This evidence of enthalpy-entropy compensation is entirely consistent with quantum-mechanical predictions of the adverse effect of water on urea dimerization. Changes in water structure thus contribute significantly to the energetics of urea dimerization in aqueous solution.

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https://doi.org/10.1016/j.bpc.2019.106175
Received 18 February 2019; Received in revised form 16 April 2019; Accepted 22 April 2019
Available online 25 April 2019

1. Introduction

The anomalous thermodynamic behavior of aqueous urea solutions was highlighted eighty years ago by isopiestic measurements at 25 °C of the chemical potential of water in sucrose, glycerol and urea solutions [1]. Whereas those measurements of the osmotic coefficient for solvent (ϕ) revealed that aqueous sucrose and glycerol solutions exhibited positive deviations from Raoult's Law, the corresponding deviations for urea solutions were negative. Concurrent estimations of ϕ from freezing point depression measurements also signified the existence of negative deviations from Raoult's Law for aqueous urea solutions [2]; and surprise was expressed soon after at the sign (negative) of the heat of dilution for aqueous urea solutions [3].

The thermodynamic nonideality of aqueous solutions of sucrose and glycerol find rational explanation on the statistical-mechanical basis of excluded volume [4,5]; and the result for urea was later attributed to solute dimerization with an association constant of 0.041 molal^{-1} at 25 °C [6]. Those Schellman deliberations triggered a resurgence of interest in the use of isopiestic measurements to characterize the temperature dependence of the osmotic coefficient for urea [7–11] – studies in which the primary objective was to provide a virial expansion in solute molality that described the osmotic coefficient over the entire range of urea solubility (0–20 molal) as well as the standard enthalpy change for dimer formation.

This communication reconsiders the results of the original isopiestic measurements on aqueous urea solutions [1] in the light of the subsequent demonstration [12,13] of the need to take into account the thermodynamic constraints under which solute chemical potential is being monitored in a given experiment.

2. Theoretical considerations

Although there is general agreement that the osmotic coefficient for urea exhibits a negative dependence upon solute molality, the quantitative interpretation of that dependence has varied because of conflicting viewpoints about its value in the limit of zero solute concentration for an ideal dimerizing solute [6,9–11]. In that regard a potential source of confusion has stemmed from a shortcoming in the usual definition of solute chemical potential, which fails to specify the thermodynamic constraints under which chemical potential is being monitored. This section therefore begins with an outline of the definition of solute chemical potential under the two commonly encountered thermodynamic constraints.

2.1. Definition of solute chemical potential

As emphasized by Hill [13], the addition of solute (component 2) to solvent (component 1) at constant temperature gives rise to one of two situations, depending upon the other thermodynamic variable that is controlled in the experiment. In partition procedures such as osmometry and equilibrium dialysis that second constraint is constancy of the solvent chemical potential, whereupon the solute chemical potential is being monitored on the molar concentration scale and the natural expression for solute chemical potential becomes

$$(\mu_2)_{T,\mu_1} = (\mu_2^o)_{T,\mu_1} + RT\ \ln\ z_2 = (\mu_2^o)_{T,\mu_1} + RT\ \ln\ (\gamma_2 C_2) \tag{1}$$

where the molar activity of solute (z_2) is most simply written as the product of the molar concentration (C_2), and the corresponding molar activity coefficient (γ_2). In terms of the usual virial expansion for osmotic pressure,

$$\frac{\Pi}{RT} = C_2(1 + \boldsymbol{B}_2 C_2 + ...) \tag{2}$$

the molar activity coefficient is related to the osmotic second virial coefficient ($\boldsymbol{B}_2$) by [14].

$$\ln \gamma_2 = 2\boldsymbol{B}_2 C_2 + ... \tag{3}$$

A particular advantage of defining solute chemical potential under the constraints of constant temperature and solvent chemical potential is that the osmotic second virial coefficient ($\boldsymbol{B}_2$) and hence the solute activity coefficient (γ_2) are rigorously and conveniently defined statistical-mechanical parameters that can be described simply in terms of physical interactions between pairs of solute molecules [4,5,14].

The more commonly encountered experimental situation entails the measurement of chemical potential under the thermodynamic constraints of constant temperature and pressure, whereupon the solute activity becomes a natural molal parameter that is related to its chemical potential by the expression

$$(\mu_2)_{T,P} = (\mu_2^o)_{T,P} + RT\ \ln\ a_2 = (\mu_2^o)_{T,P} + RT\ \ln (y_2 m_2) \tag{4}$$

in which the molal activity (a_2) is most compactly written as the product of molal concentration, $m_2 = n_2/(n_1 M_1)$ where n_2 denotes the number of moles of solute present in a mass $n_1 M_1$ of solvent, and the corresponding molal activity coefficient y_2.

Combination of the polynomial for the change in solvent chemical potential effected by the addition of solute, namely

$$\frac{(\mu_1^o)_{T,P} - (\mu_1)_{T,P}}{RTM_1} = m_2(1 + \boldsymbol{C_2} m_2 + ...) \tag{5}$$

with the appropriate form of the Gibbs-Duhem equation (Eq. [4.104] of Hill [13]);

$$\left(\frac{\partial \mu_2/kT}{\partial m_2}\right)_{T,P} = -\frac{1}{M_1 m_2}\left(\frac{\partial \mu_1/kT}{\partial m_2}\right)_{T,P} \tag{6}$$

with k the Boltzmann constant then gives.

$$\ln\ y_2 = 2\boldsymbol{C_2} m_2 + ... \tag{7}$$

as the expression for the molal activity coefficient.

Unfortunately, there are no straightforward statistical-mechanical methods for relating the molal second virial coefficient ($\boldsymbol{C_2}$) to intermolecular forces. However, the assumption of solution incompressibility (a not unreasonable approximation for aqueous solutions) allows the expression of a molal concentration in terms of molar concentration as

$$m_2 = \frac{C_2}{\rho_1(1 - M_2\bar{\nu}_2 c_2)} \approx \frac{C_2}{\rho_1}(1 + M_2\bar{\nu}_2 C_2 + ...) \tag{8}$$

where M_2 and $\bar{\nu}_2$ denote the molecular mass and partial specific volume of solute respectively; and where ρ_1 is the solvent density. It then follows that the two second virial coefficients are related by the expression [14]

$$\boldsymbol{C_2} = (\boldsymbol{B_2} - M_2\bar{\nu}_2)\rho_1 \tag{9}$$

which allows conversion of the molal second virial coefficient $\boldsymbol{C_2}$ to its osmotic second virial counterpart $\boldsymbol{B_2}$, the parameter amenable to rigorous statistical-mechanical interpretation [4].

2.2. Measurement of the osmotic coefficient

In measurements of the osmotic coefficient ϕ by the isopiestic (isotonic) procedure the magnitude of the solvent chemical potential in the vapor phase is established by including in each experiment a solution of a standard solute for which the molal concentration dependence of ϕ is known. Because the solutions of the solute of interest are also in partition equilibrium with the same vapor phase, it follows that their solvent chemical potential equates with that in the vapor phase; and hence that the magnitude of ϕ may be determined [1].

2.3. Interpretation of the osmotic coefficient

The osmotic coefficient ϕ that is determined by the isopiestic

procedure has been defined by the relationship [1]

$$\phi = \frac{(\mu_1^o)_{T,P} - (\mu_1)_{T,P}}{RTM_1 m_2} = \frac{-\ln a_1}{M_1 m_2} \tag{10}$$

which, from Eq. (5), can also be expressed as.

$$\phi_{molal} = 1 + \boldsymbol{C_2} m_2 + \ldots \tag{11}$$

with the proviso that the thermodynamic activity of solvent (a_1) is measured on the molal scale. Eq. (7) may then be used to calculate the molal activity of solute (a_2) as.

$$a_2 = m_2 \exp(2\boldsymbol{C_2} m_2 + \ldots) \approx m_2(1 + 2\boldsymbol{C_2} m_2 + \ldots) \tag{12}$$

In that regard the essentially linear dependence of the osmotic coefficient upon urea concentration for $m_2 \leq 1$ molal [1] justifies the neglect of higher-order terms in the virial expansions in Eqs. (11) and (12) describing the nonideality. Negative values of $\boldsymbol{C_2} m_2$ imply that $a_2 < m_2$ and hence signify predominance of the effects of chemical interaction over those of volume exclusion [4], the consequences of which cannot be incorporated into analysis of the $\phi - m_2$ dependence in terms of urea dimerization. We therefore need to proceed on the basis that solute dimerization is the sole source of the negative deviation from Raoult's Law.

Under conditions of thermodynamic ideality for the dimerization process, the thermodynamic activity a_2 [Eq. (12] would correspond to the sum of the molalities of monomer (A) and dimer (C): that is,

$$a_2 = m_A + m_C \tag{13a}$$

whereas the total urea concentration (m_2) is given by

$$m_2 = m_A + 2m_C \tag{13b}$$

The difference between the solute concentration (m_2) and its thermodynamic activity (a_2) thus defines the molality of dimer (m_C), for a hypothetical ideal solution of dimerizing urea. The corresponding monomer concentration (m_A) then becomes $(m_2 - 2m_C) = (a_2 - m_C)$, whereupon the magnitude of the apparent molal dimerization constant (K_2^{app}) follows as

$$K_2^{app} = \frac{m_C}{m_A^2} \tag{14}$$

Because no account is being taken of the effects of thermodynamic nonideality arising from physical interaction between molecules, the estimates of K_2^{app} for a range of urea concentrations require extrapolation to infinite dilution to obtain the true, thermodynamic dimerization constant (K_2) as the limiting value.

3. Results and discussion

As mentioned in Section 2.3, the interpretation of the osmotic coefficient ϕ in terms of Eq. (11) requires definition of the solvent thermodynamic activity (a_1) on the molal scale. We therefore begin this reappraisal of isopiestic measurements on aqueous urea solutions [1] by assessing their conformity with that requirement.

3.1. Concentration scale of the osmotic coefficient

Despite being termed the molal osmotic coefficient [11], ϕ has been determined on the basis of solvent thermodynamic activity defined [1] on the mole-fraction scale (x_1). That conclusion is evident from Fig. 1, which summarizes the reported concentration dependence of ϕ for 0.1–1.0 molal urea solutions [1]. Although the essential linearity of that dependence (■) seemingly implies conformity with its interpretation in terms of Eq. (11), such action is also dependent upon a concentration-independent value of unity for ϕ under conditions of thermodynamic ideality for a nonassociating solute. However, the reported dependence for such a system [1], shown as open symbols in Fig. 1, corresponds to the relationship

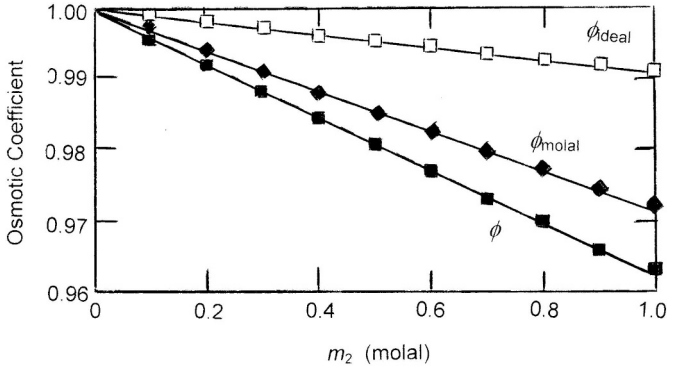

Fig. 1. Dependence of the osmotic coefficient upon molal concentration deduced from isopiestic measurements on aqueous urea solutions at 25 °C. ■, Osmotic coefficients (ϕ) taken from Table II of [1]. □, Corresponding dependence for an ideal nonassociating solute (ϕ_{ideal}) reflecting use of the mole-fraction scale for the definition of solvent thermodynamic activity [Eq. (15)]. ♦, Dependence of the revised osmotic coefficient, $\phi_{molal} = \phi - \phi_{ideal}$, with activity now defined on the molal concentration scale to conform with the constraints of constant temperature and pressure that pertain in isopiestic measurements [12,13].

$$\phi_{ideal} = \frac{\ln(1 - x_2)}{M_1 m_2} = \frac{1}{1 + M_1 m_2} \tag{15}$$

which takes into account the decrease in solvent mole-fraction $x_1 = (1 - x_2)$ with increasing solute concentration m_2. It should be noted that the molar mass of water (M_1) needs to be expressed in kg/mol to render the product $M_1 m_2$ dimensionless.

In order to accommodate the realization by Hill [12,13] that the thermodynamic activity of solvent (a_1) is being monitored on the molal concentration scale under the constraints of constant temperature and pressure that pertain in isopiestic measurements, we need to redefine the osmotic coefficient as

$$\phi_{molal} = \phi + (1 - \phi_{ideal}) \tag{16}$$

which then achieves the requirement of Eq. (4) that the molal osmotic coefficient be unity for $m_2 = 0$. The concentration dependence of this revised osmotic coefficient is also shown (♦) in Fig. 1.

3.2. Evaluation of the molal second virial coefficient

The essential linearity of the dependence of ϕ_{molal} upon the molal urea concentration (♦, Fig. 1) justifies the truncation of Eq. (11) for this system at the second virial coefficient term and hence the assignment of an estimate (± 2SD) of –0.0275 (± 0.0008) molal^{-1} to $\boldsymbol{C_2}$, the molal second virial coefficient, from the slope. Although the negative value of $\boldsymbol{C_2}$ is seemingly symptomatic of negative deviations from Raoult's Law, a slightly modified conclusion emerges upon its conversion to an osmotic second virial coefficient ($\boldsymbol{B_2}$) on the basis of assumed solution incompressibility [Eq. (9)]. Combination of the estimate of −27.5 mmolal^{-1} for $\boldsymbol{C_2}$ at 25 °C with the apparent molar volume ($M_2\bar{v}_2$) of 44.2 mL/mol for urea [11] yields an estimate of +16.7 (± 0.5) mL/mol for $\boldsymbol{B_2}$ that signifies the existence of positive deviation from Raoult's Law. However, interpretation of this estimate for $\boldsymbol{B_2}$.on the statistical-mechanical basis of excluded volume [4], $\boldsymbol{B_2} = 16\pi N_A R_2^3/3$, yields an apparent solute radius (R_2) of 0.12 nm, which is essentially the same as that of a water molecule. It must therefore be concluded that this unrealistic estimate of the solvated solute radius reflects a compromise between the opposing effects of excluded volume (which lead to positive deviation from Raoult's Law) and those of chemical interaction (solute-solute and/or solute-solvent), which give rise to negative deviations from Raoult's Law and hence decrease the magnitude of $\boldsymbol{B_2}$. These observations suffice to demonstrate the need to take into account the effects of thermodynamic nonideality arising from excluded-volume

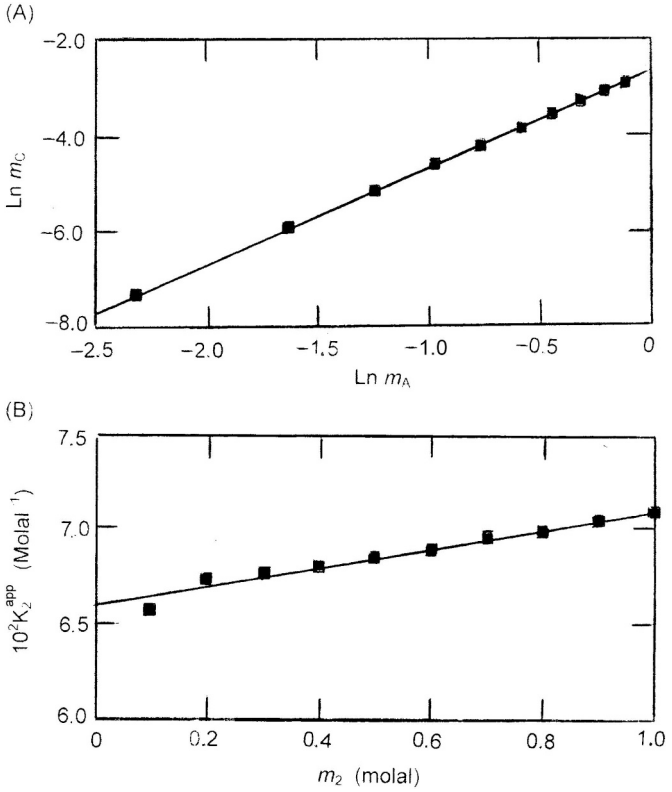

Fig. 2. Use of the osmotic coefficients (Fig. 1) to characterize the dimerization of aqueous urea at 25 °C. (A) Check on the stoichiometry of self-association by means of the logarithmic form of Eq. (14). (B) Extrapolation of the apparent dimerization constants to zero urea concentration to obtain the thermodynamic dimerization constant, K_2.

interactions on the magnitude of equilibrium constants deduced by assuming thermodynamic ideality of the chemical interaction - a conclusion already reached by Mountain and Thirumalai [16].

3.3. Evaluation of the association constant for urea dimerization

The next step of the analysis entails interpretation of the negative $\phi_{molal} - m_2$ dependence for aqueous urea solutions in terms of solute self-interaction to obtain an estimate of the dimerization constant via Eq. (14), taking into account thermodynamic ideality. Use of Eq. (12) to calculate the molal activity of urea yields a value less than m_2 because the two urea molecules comprising a dimer are being counted as a single entity. As noted in Section 2.3, the molal concentration of dimer (m_C) becomes the difference between m_2 and a_2; and that of monomer (m_A) becomes the difference between a_2 and m_C (or m_2 and $2m_C$). To confirm the identity of the oligomeric species as dimer, those species concentrations deduced from the revised Scatchard isopiestic measurements (ϕ_{molal}) at 25 °C [1] are plotted according to the logarithmic form of Eq. (14). The slope of 2.033 (± 0.005) for that dependence of ln m_C upon ln m_A (Fig. 2A) essentially reproduces the theoretical value of 2 for a monomer–dimer system, thereby eliminating the need for consideration of self-association beyond dimer over the limited urea concentration range (0.1–1.0 molal) investigated in this study to minimize the consequences of thermodynamic nonideality reflecting physical interaction between molecules. Schellman [6] accommodated the possibility of polymerization beyond dimer by introducing the concept of isodesmic indefinite self-association. However, that approach should also yield the dimerization constant because the same association constant is used for each successive monomer addition.

Calculation of the apparent dimerization constant via Eq. (14) for each [m_A, m_C] experimental point leads to the concentration dependence of K_2^{app} shown in Fig. 2B. Its extrapolation to zero solute molality yields an estimate of 0. 0.0659 (± 0.0005) molal^{-1} for K_2, the thermodynamic dimerization constant. A lower value of 0.041 molal^{-1} was deduced by Schellman [6] from the original [m_2, ϕ] data set [1] by making the approximation that the combined molalities of monomer and dimer could be substituted for their mole-fraction: i.e., that $x_{A+C} = (m_A + m_C)/(m_1 + m_A + m_C) \approx (m_A + m_C)/m_1$. His use of the resulting values of m_A and m_C in Eq. (14) to calculate the dimerization constant was later criticized by Stokes [11,15] because such action unjustifiably presumed a value of unity for ϕ for an ideal nonassociating solute. As noted above (Fig. 1), such action is justified when the osmotic coefficient is expressed on the present molal basis (ϕ_{molal}), but not when it is being monitored on the original mole-fraction basis (ϕ).

Upon adopting the convention (1) that the thermodynamic activity of water (a_1) in Eq. (10) is measured on the mole-fraction scale, Stokes evaluated the solvent mole fraction (x_1) as

$$x_1 = \exp(-\phi M_1 m_2) \tag{17a}$$

and the corresponding stoichiometric value in the absence of solute dimerization (x_1^{ideal}) is

$$x_1^{\mathrm{ideal}} = \frac{1}{1 + M_1 m_2} \tag{17b}$$

Incorporation of the mole-fraction values thereby determined into the expressions relating those solvent mole-fractions to the consequent values for the two associating species, namely

$$x_1 = 1 - x_A - x_C \tag{18a}$$

$$x_1^{\mathrm{ideal}} = 1 - x_A - 2x_C \tag{18b}$$

should then allow calculation of the mole-fraction-based dimerization constant K_2^{mf} as x_C/x_A^2. The average value of 1.80 (± 0.03) for this dimensionless constant confirms earlier estimates of 1.8 [15] and 1.78 [9] for this mean parameter. For consistency with the above treatment of ϕ_{molal}, each set of (x_A, x_C) values has been converted to the molal scale before calculation of a dimerization constant. Linear regression of those values has yielded a limiting value of 0.0329 (± 0.0005) molal^{-1} for the dimerization constant. The two-fold disparity between this Stokes estimate of the dimerization constant [15] and the current value reflects the incorrect evaluation of x_C as the difference between x_1*and* x_1^{ideal}),whereas current theoretical considerations [Eqs. (10)–(12)] show that $x_C = 2(x_1 - x_1^{\mathrm{ideal}})$. In as much as incorporation of that change into the Stokes analysis [15] doubles the magnitudes of the calculated dimerization constants, the disparity is thereby removed.

3.4. Energetics of dimerization in aqueous urea solutions

The standard free energy change for urea dimerization (ΔG^o) is readily calculated from the above value of K_2 on the basis that $\Delta G^o = -RT \ln K_2$: a value of 6.74 (± 0.02) kJ/mol is obtained. Further characterization of the energetics of urea dimerization requires an independent estimate of the standard enthalpy change (ΔH^o) at the same temperature. On the grounds that urea is monomeric at infinite dilution, it follows [6,11] that knowledge of the relative heat content, $\Phi_L = \Phi_H - \Phi_H{}^0$ with $\Phi_H{}^0$ the limiting value, allows estimation of the standard enthalpy change for urea dimerization (ΔH^o) as

$$\Phi_L = \Delta H^o m_C/m_2 \tag{19}$$

which on the basis of Eqs. (13a) and (13b) may be written as

$$\Phi_L = \Delta H^o(1 - a_2/m_2) \tag{20}$$

As noted by Schellman [6], values of the relative heat content are available from the empirical relationship reported by Gucker and Pickard [3] but now modified to express Φ_L in J/mol,

$$\Phi_L = -359.5m_2 + 28.53m_2^2 - 0.1913m_2^3 \tag{21}$$

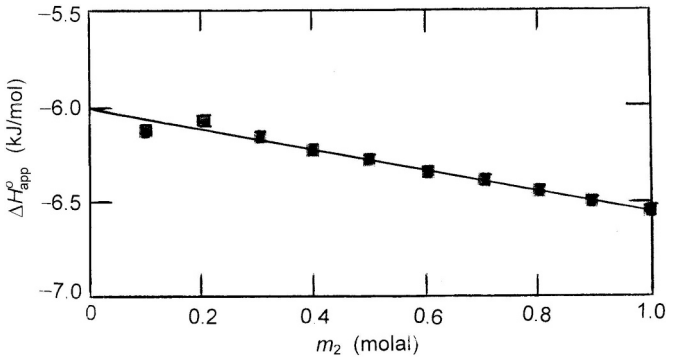

Fig. 3. Evaluation of the standard enthalpy change (ΔH^o) for urea dimerization at 25 °C by extrapolating apparent values calculated from Eq. (22) to zero solute concentration in order to eliminate effects of thermodynamic nonideality arising from excluded volume interactions.

Substitution of $(1 + 2C_2m_2)$ for a_2/m_2 [Eq. (12)] and $(\phi - 1)$ for C_2m_2 [Eq. (11)] then allows the replacement of $(1 - a_2/m_2)$ by $2(1 - \phi_{molal})$ in Eq. (20), whereupon an apparent magnitude of ΔH^o for each $[m_2, \Phi_L]$ data point can be calculated from the expression.

$$\Delta H^o_{app} = \frac{\Phi_L}{2(1 - \phi_{molal})} \tag{22}$$

Extrapolation of those estimates of ΔH^o_{app} to zero urea concentration to eliminate the effects of thermodynamic nonideality arising from excluded volume interactions (Fig. 3) yields a standard enthalpy change of −6.04 (± 0.05) kJ/mol.

Its combination with the above value of ΔG^o into the Gibbs-Helmholtz equation then gives an estimate of −12.78 (± 0.07) kJ/mol for the product of T and ΔS^o (the standard entropy change for urea dimerization).

An obvious outcome of these deliberations is the extremely large negative entropic contribution to the energetics of urea dimerization at 25 °C. Although some entropic disadvantage must emanate from the restricted relative movement of the two urea monomers comprising a dimer, the magnitude of the calculated $T\Delta S^o$ contribution to ΔG^o signifies additional sources for the observed enthalpy–entropy compensation. Because the hydroxyl groups of water are potential contenders for hydrogen bond formation with either urea and/or other water molecules, the large negative entropic contribution to the standard free energy of dimerization is attributed to restricted movement of water molecules in the vicinity of urea molecules through their involvement in solute–solvent and possibly modified solvent–solvent interactions. Such interpretation is entirely consistent with theoretical predictions of the chemical structure of the aqueous urea dimer [17–20], a notable feature of which is the attachment of water molecules to the urea dimer by hydrogen bonding. It is interesting to note that such changes in the hydration state of urea itself have been shown to play a minimal role in the denaturation of proteins by urea [21].

An extremely notable example of support for the concept that the negative entropic contribution is a consequence of solvent interactions comes from the results of a quantum-mechanical investigation into the adverse effect of water on urea dimerization [20], which is attributed to the existence of urea–water and water–water hydrogen bonds in the dimer at higher degrees of hydration. Any transiently formed cyclic dimer with two imino-carbonyl hydrogen bonds (predicted to exist in the absence of other factors) is rapidly hydrated (within a few picoseconds) to an equally transient structure with the two urea molecules linked by a single imino-carbonyl hydrogen bond. During this dissociation process the monomers are surrounded by a shell of water molecules that are hydrogen-bonded either to the urea or to other water molecules and water molecules for transient bridges between the two monomers. Quantitative assessment of the effect on the dimerization constant of urea–water and water–water hydrogen bonds would require impossibly detailed calculations to average effects over the statistical properties of a large ensemble of temporal trajectories of two urea molecules interacting with clusters of many water molecules.

On the basis of that quantum-mechanical investigation the starting point for urea dimerization in aqueous solution would entail rearrangements of the structured water shells surrounding two monomers to allow the substitution of an imino–carbonyl bond for a hydroxyl-carbonyl bond – a process that would undoubtedly also entail further rearrangement of water structure within the shell surrounding the newly formed urea dimer. Even in the event that the dimer with two imino-carbonyl hydrogen bonds were to form, that change would again only entail the substitution of a solute-solute hydrogen bond for one involving solute and solvent. Despite the fact that the interaction being monitored in aqueous solution by isopiestic measurements is urea dimerization [6], changes in solvent structure dominate the reaction energetics.

4. Conclusions

A major goal of this investigation has been to update the analysis of thermodynamic nonideality that affects isopiestic measurements of solvent thermodynamic activity [1]. Specifically, the experimentally determined osmotic coefficient (ϕ) has been redefined to accommodate the fact that solvent activity a_1 being monitored is a molal parameter because of the constraints of constant temperature and pressure that pertain in these experiments [12,13]: it was previously expressed on the mole-fraction basis [1]. Application of the revised approach to isopiestic [1] and heat of dilution [3] measurements on aqueous urea solutions has amended the quantitative detail but confirmed the existence of a large negative entropy contribution to the energetic of urea dimerization [6]. Because the full significance of that evidence for enthalpy-entropy compensation in the dimerization energetic was not appreciated at the time, that aspect has also been highlighted.

The relatively weak dimerization observed in aqueous urea solutions reflects competition between the favorable enthalpic contribution to ΔG^o arising from hydrogen bonding between the carbonyl and imino groups of the solute and a large unfavorable entropic contribution that stems from solute-solvent and solvent-solvent interactions in the immediate vicinity of the solute molecule. A similar situation pertains to the dimerization of *N*-methylformamide in aqueous solution – a system for which total enthalpy-entropy compensation leads to temperature independence of the standard free energy change [22]. The concept of changes in water structure is, of course, central to our understanding of the enthalpy-entropy compensation that is observed in the binding of hydrophobic and charged ligands to macromolecular solutes [23–27]. This investigation emphasizes the fact that solvent structure perturbation can also play an important role in modulating the energetics of small solute interactions as well as those involving macromolecules.

The days of regarding water as an inert solvent are clearly over - a situation that spells an end to any remaining hopes that the effects of thermodynamic nonideality in the crowded molecular environment of the biological cell might be predicted almost exclusively on the statistical-mechanical basis of excluded volume [4], also termed molecular crowding [28]. The fact that those statistical-mechanical predictions based on the effects of physical solute-solute interactions can be overturned by the superimposition of chemical solute-solute interactions has always been recognized as a potential weakness of the approach. Here we have drawn attention to the existence of solute-solvent and solvent-solvent interactions as a far more substantial barrier to successful quantification of the effects of thermodynamic nonideality in physiological situations. However, the use of those theoretical predictions based on the effects of molecular crowding [28–30] remains a powerful qualitative means for examining potential consequences of thermodynamic nonideality in the crowded cellular environment of the biological cell [31,32].

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