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Non-polar solutes in water and in aqueous solutions of protein denaturants. Modeling of solution and transfer processes

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Abstract

A simple molecular model for the thermodynamic behavior of non-polar solutes in water and in aqueous solutions of protein denaturants is presented. Three contributions are considered: (i) combinatorial arising from the mixing process, (ii) interactional characterizing the molecular interactions occurring in the mixture and (iii) a contribution originating from the structural changes occurring in the first shell of water molecules around the solute. The latter is modeled assuming that water molecules in contact with the solute are involved in a chemical equilibrium between two states. The model describes well the temperature and denaturant concentration dependences of the Gibbs energies of solution and transfer for benzene, toluene and alkanes in water and aqueous solutions of urea and guanidine hydrochloride. Model parameters are physically meaningful, allowing a discussion of the molecular interactions involved. A preferential solvation of the solute by the denaturant is found. However, the non-polar solute-denaturant interaction is not specific, i.e. leading to a distinct chemical entity. Urea and guanidine hydrochloride are non-polar solubilizing agents because their interactions with the solute are less unfavorable than those between water and the solute. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Native proteins are routinely unfolded at constant temperature using aqueous solutions of urea or guanidine hydrochloride. Denaturation with these chemicals is one of the primary ways of measuring the conformational stability of proteins [1]. The mechanism involved in protein denaturation is not well established. It is not clear if denaturants act indirectly through effects on the water solvent, or directly by favorable interactions (solvation) of non-polar, polar and ionic groups or by binding, via hydrogen bonding, with the amide units in the polypeptide chain [2–11]. Furthermore, it is also not clear which is the relative importance of each of these effects, i.e. whether one of them is dominant or all of them are significant. The complexity of protein denaturation makes it necessary to dissect this multi-faceted phenomenon into simple components, so that some of its aspects can be clearly understood. Within this context, we study in this work the molecular interactions occurring between non-polar substances and denaturants in aqueous media.

Upon denaturation, the non-polar chemical groups in the protein interior are exposed to the aqueous media. Hence, thermodynamic studies involving small and sparingly soluble non-polar substances in water and in (water + denaturant) solutions are of primary importance. Some thermodynamic properties of aqueous solutions of urea and guanidine hydrochloride (hereon, denoted as guanidine) and their ability to solubilize non-polar entities have been studied previously [12–15]. Recently, we have reported experimental infinite dilution activity coefficients γ_1^∞ for two non-polar solutes (benzene and toluene) in water and in (water + urea) or (water + guanidine) solutions in the 273–323 K temperature interval and at four denaturant concentrations (0.5–5.0 mol dm^{-3}), covering the range where proteins undergo chemical denaturation [16]. Activity coefficients are directly related to the Gibbs energy through $\ln\gamma_1^\infty = \Delta_{sol}G_1^\infty/RT$ where $\Delta_{sol}G_1^\infty$ is the Gibbs energy of solution that refers to the process where the solute is transferred from the pure liquid state to water at infinite dilution. If the non-polar/water mutual solubilities are small (solute mole fraction, x_1^{sol}, less than 0.001) then the reciprocal value of this mole fraction approximates the infinite dilution activity coefficient, i.e. $\gamma_1^\infty = 1/x_1^{sol}$. Another relevant quantity, which is also easily obtained from the measured values of γ_1^∞, is the transfer Gibbs energy, $\Delta_{tr}G_1^\infty$, which characterizes the process where the solute is transferred from water to a (water + denaturant) mixture. Transfer Gibbs energies have been used to estimate, through an additivity scheme, the Gibbs energy for the denaturation process [17,18].

In Hovorka et al. [16] the data were analyzed using a classical thermodynamic scheme which allowed the derivation of other thermodynamic quantities (enthalpies, entropies and heat capacities) and a detailed discussion, at the macroscopic level, of the several observed trends. In this work, one of our main goals is to study in detail the non-polar solute–denaturant interactions. In particular, we are interested in establishing whether or not the solute–denaturant interactions are strong enough so that this interaction can be classified as specific and hence leading to the formation of a solute–denaturant complex or to a preferential binding. For this purpose, we present a simple molecular model for the thermodynamic behavior of non-polar solutes in water and in (water + denaturant) mixtures. We apply this model to our data for benzene and toluene [16] and to literature thermodynamic data for *n*-alkanes [15,19,20]. The model parameters have clear physical significance and allow a detailed discussion, at the microscopic level, of the experimental results. We also apply to the available data the modified hydration shell hydrogen bond model reported in [21–23], testing its performance, comparing its results with those of the present work and examining the physical considerations upon which this model is based.

2. Hydration shell chemical equilibrium model

In this section a simple molecular model is presented for infinite dilution thermodynamic properties of a non-polar liquid solute (1) in a mixed binary solvent composed of water (2) and a substance called modifier (3). The case of a hydrophobic solute in pure water solvent is then comprised as a special case. The modifier is an additive, such as the protein denaturants studied in this work, modifying the aqueous environment where the non-polar solute is placed at infinite dilution. The model is developed for non-ionic solutions; then applying it when the modifier is guanidine ignores that this denaturant is an electrolyte. The derivation centers on the Gibbs energy of solution $\Delta_{sol}G_1^{\infty}$; the Gibbs energy of transfer $\Delta_{tr}G_1^{\infty}$ and related functions (enthalpy, entropy, heat capacity) being then readily procurable by standard procedures. The model stems from the widely accepted idea that water molecules in direct contact with the solute are, because of their spatial position, in a different condition from those in the bulk. Since 1945 when this idea was first introduced by Frank and Evans [24], a number of conceptual models have been presented to describe and visualize the formation of this structurally altered first hydration shell [25–28]. This idea is combined here with the simplest treatments of molecular thermodynamics, yet resulting in a physically meaningful correlation/interpretation scheme, that hereon will be referred as the hydration shell chemical equilibrium (HSCE) model.

The model considers $\Delta_{sol}G_1^{\infty}$ being composed of three contributions

$$\Delta_{sol}G_1^{\infty} = \Delta G^{comb} + \Delta G^{int} + \Delta G^{alt} \tag{1}$$

which originate, respectively, from combinatorial effects, molecular interactions and the structural alteration of water molecules in the first solvation shell around the solute. The assumption that only the first hydration layer is affected by the solute receives support from computer simulations [29]. The three contributions depend on molecular geometry of the species involved, so we will introduce first the respective molecular geometrical parameters.

As in the UNIQUAC/UNIFAC excess Gibbs energy models [30,31], volume and contact area characteristics, R_i and Q_i, of each component are used to describe the molecular geometry. They are defined as the molecule van der Waals volume V_i^w and area A_i^w [32] relative to those of a standard segment [30].

$$R_i = V_i^w/V_{ref}^w; V_{ref}^w = 1.517 \cdot 10^{-3}\ \mathrm{m^3\,mol^{-1}} \tag{2}$$

$$Q_i = A_i^w/A_{ref}^w;\ A_{ref}^w = 250 \cdot 10^3\ \mathrm{m^2\,mol^{-1}} \tag{3}$$

For solvent components, however, rather than the total contact area A_i^w of the molecule, essential is the area $\overline{A_i^w}$ the molecule occupies on the surface of the solvation shell, and through which the solvent and solute molecules are in contact. We determined this contact area for water and the two protein denaturants studied in this work as indicated in Fig. 1. It is considered here that these solvent molecules interact with a non-polar hydrocarbon solute through their hydrogen atoms facing the hydrocarbon surface, for water the involvement being assumed just for one of its hydrogen atoms. Such a constellation receives support from recent quantum mechanical ab initio gas-phase calculations and molecular simulation studies [33]. As seen from Fig. 1, $\overline{A_i^w}$ is approximated by the trail area of the hydrogen atoms on a planar contact surface. In our calculations we used a value of 12 nm for the van der Waals radius of the hydrogen atom, and the interatomic distances and angles reported for urea from neutron diffraction studies [34]. Considering that the chemical structure of guanidinium cation is analogous to that of urea, we adopted for this

Table 1
Geometric parameters of components

Component	R	Q	$\bar{Q}$
Benzene	3.1878	2.400	–
Toluene	3.9228	2.968	–
Methane	1.1278	1.156	–
Ethane	1.8022	1.696	–
Propane	2.4766	2.236	–
Butane	3.1510	2.776	–
Pentane	3.8254	3.316	–
Hexane	4.4998	3.856	–
Cyclohexane	4.0464	3.240	–
Water	0.9200	1.400	0.109
Urea	2.1408	2.486	0.244
Guanidine hydrochloride	3.1277	3.234	0.244

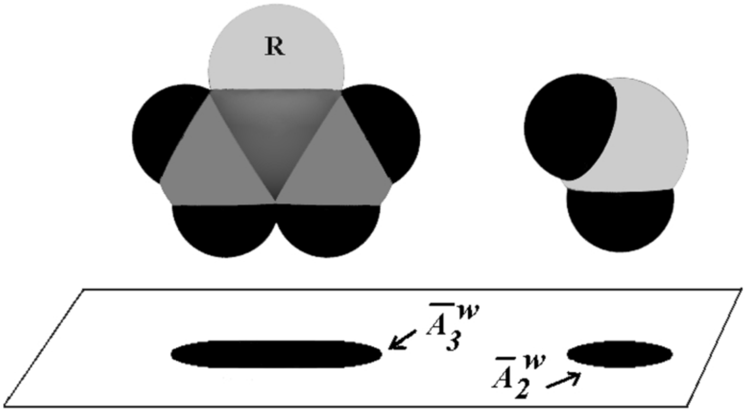

Fig. 1. Diagrammatic representation of the areas $\bar{A}_i^w$ that urea ($R = \mathrm{CO}$), guanidinium ion ($R = \mathrm{NH}_2^+$) and water occupy on the surface of the solvation shell.

cation the $\bar{A}_i^w$ value for urea. The values for $\bar{Q}_i$, normalized by the area of the standard segment, are given in Table 1, together with the other structural parameters of the components.

To describe ΔG^{comb} and ΔG^{int}, a classical approach based on the rigid lattice theory and Langmuir's concept of surface–proportional molecular interactions is employed. For the combinatorial contribution we use the Flory–Huggins expression:

$$\Delta G^{comb} = RT\left(1 + \ln\frac{R_1}{R_2x_2 + R_3x_3} - \frac{R_1}{R_2x_2 + R_3x_3}\right) \tag{4}$$

where x_2 and x_3 are the mole fractions of water and modifier, respectively. Note that ΔG^{comb} is negative and, since $R_1 > R_2$ and $R_3 > R_2$, in pure solvent water and dilute aqueous modifier solutions, it increases in absolute magnitude with increasing solute size and decreases with increasing modifier concentration.

To construct the interactional term, we will balance pair potential energy ($\Delta G^{int} = \Delta U^{int}$) for the following three hypothetical steps involved in the dissolution process ($\Delta U^{int} = \Delta U^{I} + \Delta U^{II} + \Delta U^{III}$): (I) breaking interactions in the pure liquid solute to obtain free solute molecules, (II) breaking interactions in the mixed solvent to open cavities for the solute molecules and (III) establishing interactions between the solute and the mixed solvent when the solute molecules are placed into the cavities. Let σ_{ij} stand for the molar i–j pair interaction energy per standard segment surface ($\sigma_{ij} < 0$); for brevity, hereon this quantity will be called pair interaction energy. The energy required to get one mole of free solute molecules is then simply

$$\Delta U^{I} = -Q_1\sigma_{11}/2 \tag{5}$$

Similarly, in the second step, the energy to open cavities that would accommodate this one mole of solute in the (2 + 3) mixed solvent can be written as:

$$\Delta U^{II} = -Q_1\bar{\sigma}_{(2+3)}/2 \tag{6}$$

where $\bar{\sigma}_{(2+3)}$ denotes the mean energy of the broken pair interactions. It can be readily shown that $\bar{\sigma}_{(2+3)}$ is given by the well-known quadratic mixing rule:

$$\bar{\sigma}_{(2+3)} = \xi_2^2\sigma_{22} + \xi_3^2\sigma_{33} + 2\xi_2\xi_3\sigma_{23} \tag{7}$$

with ξ_2 and ξ_3 being the fractions of the cavity surface occupied by water and modifier, respectively. These surface fractions can be calculated from

$$\xi_2 = \frac{\bar{Q}_2x_2}{\bar{Q}_2x_2 + \bar{Q}_3x_3}\ ;\ \xi_3 = 1 - \xi_2 \tag{8}$$

where $\overline{Q}_2$ and $\overline{Q}_3$ are the portions of the cavity surface taken by one molecule of the respective type. As discussed above, the values of $\overline{Q}_i$ are regarded as special geometrical parameters of solvent molecules. Considering now the third step, the drop in potential energy upon forming the interactions between the solute and the mixed solvent can be expressed as the product of Q_1 and $\bar{\sigma}_{(12,13)}$, the mean pair interaction energy of the solute with the cavity surface, i.e.:

$$\Delta U^{III} = Q_1 \bar{\sigma}_{(12,13)} \tag{9}$$

where $\bar{\sigma}_{(12,13)}$ is given by:

$$\bar{\sigma}_{(12,13)} = \xi_2 \sigma_{12} + \xi_3 \sigma_{13} \tag{10}$$

Summing up the energy changes for the three steps, the total interactional contribution takes the form:

$$\Delta G^{int} = Q_1 \left[\Delta_{12} + \xi_3 (\Delta_{13} - \Delta_{12} - \Delta_{23}) + \xi_3^2 \Delta_{23} \right] \tag{11}$$

which is written in terms of the interchange energies Δ_{ij} defined as:

$$\Delta_{ij} \equiv \sigma_{ij} - \frac{1}{2}(\sigma_{ii} + \sigma_{jj}) \tag{12}$$

Note that ΔG^{int} is proportional to the solute contact area and, since for hydrophobic solutes Δ_{12} is anticipated to be clearly dominant ($\Delta_{12} \gg 0$), ΔG^{int} must be positive and large.

To express ΔG^{alt}, the last term in Eq. (1) emerging from the structural alteration of water molecules due only to the presence of the solute, we adopted the chemical equilibrium approach. It is assumed that this alteration can be formally described by the chemical reaction

$$\mathit{Water}\ (\mathit{normal}) \Leftrightarrow \mathit{Water}\ (\mathit{altered}) \tag{13}$$

without virtually specifying neither a concrete molecular mechanism of this alteration, nor a particular spatial arrangement of water molecules in both initial and final states. We assume that only water molecules in the first shell around the solute undergo the alteration represented by Eq. (13), all water molecules in the bulk being considered intact. The alteration reaction can be characterized by an equilibrium constant K whose temperature dependence is given by:

$$\ln K = \ln K_{298} + \frac{\Delta H^0}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right) \tag{14}$$

where K_{298} is the value of K at 298.15 K and $\Delta H^{\cdot}$ is the temperature independent standard enthalpy change of the alteration. We further assume that the interactions of the normal water and the altered water molecules with their surroundings (solute and modifier) are the same and that the normal water/altered water interchange energy is zero. Under the framework of the rigid lattice theory, it can be then readily shown that the activity coefficients of the altered water species and the normal water species in the solvation shell have the same value, independently of the extent of alteration. Thus, the equilibrium condition for the alteration reaction can be written simply as

$$K = x_2^{alt} / x_2^{norm} \tag{15}$$

where x_2^{alt} and x_2^{norm} are the mole fractions of the altered and normal water species in the first solvation shell. Since $x_2^{alt} + x_2^{norm} = x_2$,

$$x_2^{alt} = x_2 \frac{K}{1+K} \tag{16}$$

$$x_2^{norm} = x_2 \frac{1}{1+K} \tag{17}$$

Let further n_2, n_2^{alt} and n stand, respectively, for the number of moles of water, altered water and the total number of moles in the first solvation shell, each per one mole of the solute. The change of the Gibbs energy accompanying the alteration reaction is then:

$$\Delta G^{alt} = n_2^{alt} \Delta G^0 + n\left[G^M(x_2^{alt}) - G^M(x_2^{alt} = 0) \right] \tag{18}$$

where $\Delta G^{\circ} = -RT\ln K$ is the standard Gibbs energy of the reaction and

$$G^M(x_2^{alt}) = RT(x_2^{alt}\ln x_2^{alt} + x_2^{norm}\ln x_2^{norm} + x_3\ln x_3) \tag{19}$$

and

$$G^M(x_2^{alt}=0) = RT(x_2\ln x_2 + x_3\ln x_3) \tag{20}$$

are the molar Gibbs energies of mixing for the equilibrium and the initial mixture, respectively, in the first solvation shell. Taking into account Eqs. (16) and (17), ΔG^{alt} can be simplified to:

$$\Delta G^{alt} = -n_2 RT\ln(1+K) \tag{21}$$

which on expressing n_2 through surface quantities:

$$n_2 = \left(Q_1/\overline{Q}_2\right)\xi_2 \tag{22}$$

takes the following final form:

$$\Delta G^{alt} = -\left(Q_1/\overline{Q}_2\right)\xi_2 \; RT\ln(1+K) \tag{23}$$

Note that ΔG^{alt} is proportional to the solute contact area Q_1 and is always negative. According to Eq. (23), water molecules in direct contact with the solute accommodate their own structure or organization to decrease the total free energy of the system. ΔG^{alt} is then of opposite sign to that for the interactional contribution, Eq. (11), and hence in the HSCE model the Gibbs energy of solution given by Eq. (1) is the result of the balance between contributions of different signs.

Another model to describe the structural changes occurring to water molecules in the first solvation shell around a hydrophobic solute has been presented [35]. This model considers the fact that the water molecules around the solute undergo a relaxation process described by a two-state model. In the Appendix A, it is shown that the Gibbs energy change for this relaxation process is equivalent to the ΔG^{alt} in the HSCE model described above and the relations linking both models are given.

In the development of the ΔG^{int} and ΔG^{alt} contributions outlined above, we have assumed that the composition of the mixed solvent in the first solvation shell around the solute is the same as in the bulk; in other words we assumed random mixing. However, since the interaction of the solute with the denaturant is expected to be more favorable than that with water, a preferential solvation of the solute by the denaturant seems to be more likely the case. In order to account for this preferential solvation, we employed the local composition concept of Wilson [36] that has served as a basis for a number of successful excess Gibbs energy equations. Accordingly, the local surface fractions of water and the modifier in the first solvation shell are:

$$\xi_2 = \frac{\overline{Q}_2 x_2}{\overline{Q}_2 x_2 + \overline{Q}_3 x_3 \exp[-(\sigma_{13}-\sigma_{12})/RT]}; \quad \xi_3 = 1 - \xi_2 \tag{24}$$

rather than those given by Eq. (8). A non-zero $\sigma_{13}-\sigma_{12}$ value induces non-randomness [for $\sigma_{13}-\sigma_{12}=0$, Eq. (24) reduces to Eq. (8)], and its sign indicates the component of the mixed solvent that preferentially solvates the solute. The use of Eq. (24) implies the appearance of an additional parameter, namely $\sigma_{13}-\sigma_{12}$. With this in mind, comparison between the results with Eq. (8) and Eq. (24) might serve as a probe of the feasibility of preferential solvation by the modifier. It should be noted here that a meticulous incorporation of the local composition idea into the HSCE model scheme would require the consideration of an additional interactional contribution emerging from the change in the number of 2–3 pair contacts in the solvation shell, with respect to the bulk. This lengthy interactional term is omitted here, as the version of the model incorporating it has been found not to offer any improvement.

The HSCE model described above consists of Eqs. (1), (4), (11) and (23). If Eq. (24) is used, the model involves six adjustable parameters that must be evaluated from experimental data. Four of these parameters ($\Delta_{12}, \Delta_{13}, \Delta_{23}$ and $\sigma_{13}-\sigma_{12}$) characterize pair interactions and the remaining two (K_{298}, ΔH^0) describe the structural alteration of water in the first solvation shell. If Eq. (8) is employed, the total number of parameters is five. The model equation for $\Delta_{sol}G_1^\infty$ of a hydrophobic solute in pure water solvent is a special case ($x_3 = 0$), and contains three parameters namely Δ_{12}, K_{298}, ΔH^0; these three parameters can be evaluated from the (solute + water) binary mixture data.

The model equation for the transfer Gibbs energy is obtained from:

$$\Delta_{tr}G_1^\infty = \Delta_{sol}G_1^\infty(x_3) - \Delta_{sol}G_1^\infty(x_3 = 0) \qquad (25)$$

and the number of parameters to be adjusted to experimental transfer data reduces in this case to five ($\Delta_{13} - \Delta_{12}$, Δ_{23}, $\sigma_{13} - \sigma_{12}$, K_{298},ΔH^0) or four if Eq. (8) is used. The respective equations for enthalpies, entropies, and heat capacities are not explicitly given here as they can be obtained from Gibbs energies by standard thermodynamic procedures.

3. Results and discussion

3.1. Evaluation of the HSCE model parameters

In this section the HSCE model parameters are evaluated using experimental data. For the case of benzene and toluene, we used Gibbs energies of solution in pure water and in aqueous solutions of urea and guanidine as a function of temperature and denaturant concentration [16]. We also evaluated the model parameters for the alkanes using literature data [15,19,20]. In order to minimize correlations between model parameters we adopted the following policy (*i*) employ a data base as broad and good quality as possible, (*ii*) split evaluation of parameters into gradual steps, reducing thus the number of those evaluated simultaneously and (*iii*) keep the number of parameters to be adjusted as low as possible by fusing some of them by plausible assumptions, e.g. adopt the same $\sigma_{13} - \sigma_{12}$ value for a solute series in a given cosolvent. Although the correlation between model parameters can never be eliminated completely, we believe that the sound parameter evaluation policy ensures their meaningful molecular-level interpretation given in Section 3.2.

For alkylbenzenes, the parameters $\ln K_{298}$, ΔH^0 and Δ_{12} were obtained through a simultaneous fitting of benzene and toluene pure water solvent data [16], the results being displayed in Table 2. It was found that the data are well fitted with common $\ln K_{298}$ and ΔH^0 and individual Δ_{12} values. Fig. 2 shows the good quality of the fitting. For the alkane series, we used available pure water solvent data for those alkanes for which direct data for the solution process is available [19,20] and no recalculation from the gaseous to liquid standard state is needed. Due to the low number of experimental points available, we took advantage of the fact that the HSCE model equations for the solution entropy $\Delta_{sol}S_1^\infty$ and heat capacity $\Delta_{sol}C_{P1}^\infty$ do not comprise the Δ_{12} parameter and fitted $\ln K_{298}$ and ΔH^0 to these properties. As indicated in Table 2, common $\ln K_{298}$ and ΔH^0 values for the alkane series were found to give a satisfactory fit for the data. Subsequently, Δ_{12} was fitted as a unique parameter to solubility x_1^{sol} data [20], its value being also

Table 2
The HSCE model parameters fitted to binary non-polar solute (1) + water (2) data

Solute series	$\ln K_{298}$	$\Delta H^0/\mathrm{J \cdot mol^{-1}}$	Solute(1)	$\Delta_{12}/\mathrm{J \cdot mol^{-1}}$
Alkylbenzenes[a]	−1.0434	−6214	Benzene	16 190
			Toluene	15 980
Alkanes	−0.8808[b]	−6694[b]	nC_5, nC_6, cC_6	17 850[c]

[a] Fitted to γ_1^∞ from [16], $s_{rel} = 1.3\%$.

[b] Fitted to $\Delta_{sol}S_1^\infty$ from [19,20] and $\Delta_{sol}C_P^\infty$ from [19], $s_{rel} = 4.4\%$.

[c] Fitted to x_1^{sol} from [20], $s_{rel} = 25\ \%$.

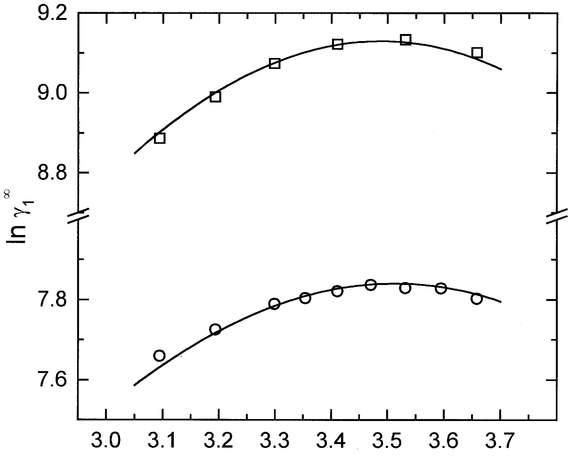

Fig. 2. The HSCE model fit (lines) to the experimental limiting activity coefficients $\ln\gamma_1^\infty$ of benzene (○) and toluene (□) in water as a function of temperature. This simultaneous fit produced the parameters reported in Table 2. The experimental data are from [16].

reported in Table 2. Although the relative standard deviation of this fit is quite large (see Table 2), it is comparable to the scatter of the reported experimental solubilities for these highly hydrophobic solutes.

The HSCE model parameters describing the solute–denaturant and water–denaturant interactions can be evaluated by employing the solution Gibbs energies or, equivalently, the transfer Gibbs energies. If Eq. (8) is utilized, these parameters are Δ_{13} and Δ_{23} and, if Eq. (24) is used, there is also the additional $\sigma_{13} - \sigma_{12}$ parameter. The performances of Eq. (8) and Eq. (24) are shown in Fig. 3 in the case of benzene in aqueous solutions of urea and guanidine, the experimental data being from [16]. For all the systems studied in this work, the standard deviations of the fit are lower (by a factor of 1.5–2) in the case of Eq. (24) than in Eq. (8). The difference between these standard deviations is statistically significant. For Eq. (24) to be considered superior to Eq. (8), it is also a requirement that the parameter $\sigma_{13} - \sigma_{12}$ is different from zero with statistical significance. This condition is fulfilled for all solutes. It appears then that consideration of preferential solvation, i.e. Eq. (24), gives a better rendering of the experimental data. The excellent fits achieved with Eq. (24) are illustrated in Fig. 4 with the data [16] for toluene in aqueous solutions of urea and guanidine.

For both alkylbenzenes, the parameters are reported in Table 3. For each denaturant, it was found that the data were well fitted with individual values for Δ_{13} and a common $\sigma_{13} - \sigma_{12}$ value for both solutes. In the case of alkanes in aqueous solutions of urea or guanidine, transfer Gibbs energy data are available only for the lower alkanes, methane to butane [15]. In order to describe this experimental data, it was necessary to introduce a solute size dependence for the $\Delta_{13} - \Delta_{12}$ parameter. Within the framework of the rigid lattice theory, upon which the HSCE model is based, solute–size dependences for any of its parameters is not justified. Hence, the analytical form of the $(\Delta_{13} - \Delta_{12})$ alkane–size dependence in Table 3 is arbitrary. Note that this dependence is important only for small size alkanes. As shown in Fig. 5, the use of Eq. (24) gave again better results than Eq. (8). In these fittings, the water–denaturant interchange energy parameter Δ_{23} was obtained from the alkylbenzenes series data. As with the alkylbenzenes series, a common $\sigma_{13} - \sigma_{12}$ value for all alkanes, but particular for each denaturant, gave good results. The alkane series parameters are given in Table 3.

3.2. A molecular view

In the previous section, we showed that despite of the simplicity of the HSCE model, it fits the experimental data to nearly experimental uncertainty. At the correlation level, the HSCE model is able to represent the data with less parameters than those needed using a classical thermodynamic scheme [16]. At the interpretation level, the HSCE model is a useful tool to explain, from the molecular point of view, the behavior of nonpolar solutes in water and in aqueous solutions of modifiers such as the protein denaturants studied here. This stems from the physical significance of the model parameters and their plausible values that we now discuss.

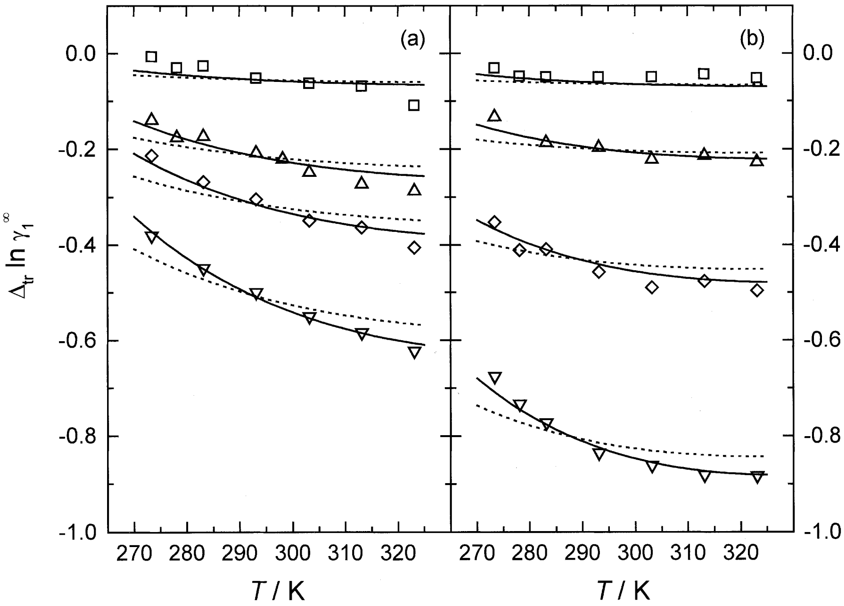

Fig. 3. The HSCE model fits to the experimental transfer limiting activity coefficients $\Delta_{tr}\ln\gamma_1^\infty$ of benzene in aqueous solutions of (a) urea and (b) guanidine as a function of temperature, at several denaturant molar concentrations (mol/dm^3): □, 0.5; △, (a) 2.0 and (b) 1.5; ◇, 3.0; ▽, 5.0. $\Delta_{tr}\ln\gamma_1^\infty$ refers to solute transfer from pure solvent water to an aqueous solution of denaturant [see Eq. (25)]. Full lines were obtained using Eq. (24) and dotted lines using Eq. (8). These simultaneous fits produced the parameters reported in Table 3 [for the Eq. (24) case]. The experimental data are from [16].

3.2.1. Non-polar solutes in pure water solvent

The K_{298} and $|\Delta H^0|$ parameters in Table 2 are larger for alkanes than for the alkylbenzenes. Clearly, the alteration of water molecules induced by an alkane is stronger in the sense that more water molecules are altered in the first solvation shell around the solute, and that this alteration process is energetically more efficient than when the solute is an alkylbenzene. For both series of solutes, the Δ_{12} parameters in Table 2 take large positive values, expressing the interactional animosity or dislike between non-polar substances and water, a dislike that is less pronounced for alkylbenzenes. This agrees with the finding that alkylbenzenes have been found to form weak hydrogen bonds with water [37–40]. On the other hand, in Table 2 Δ_{12} is bigger for benzene than for toluene. This is an inconsistency that probably arises from the simplicity of the model.

In the HSCE model, the solution Gibbs energy is the result of the balance between competing contributions as shown in Fig. 6a for the case of toluene in water. On the one hand there is a large positive interactional contribution ΔG^{int} [Eq. (11)] disfavoring solubility. On the other hand, there are two negative contributions to the total solution Gibbs energy, the combinatorial ΔG^{comb} [Eq. (4)] which is small and the large ΔG^{alt} [Eq. (23)] emerging from the ability of water molecules around the solute to change their structure, both contributions promoting solubility. As indicated in Fig. 6a the interactional contribution is dominant, overcoming the water/alteration and combinatorial contributions, the net result being the well-known experimental positive solution Gibbs energy shown in Fig. 6b. In the framework of the HSCE model, the hydrophobicity of non-polar solutes is then caused by the large positive value for the solute–water interchange energy (Δ_{12}), that in turn is due to the high cohesive energy of water [σ_{22} in Eq. (12)]. Simply, the attraction of the water molecules to each other is much greater than their attraction to the solute molecules and the attraction of the solute molecules to each

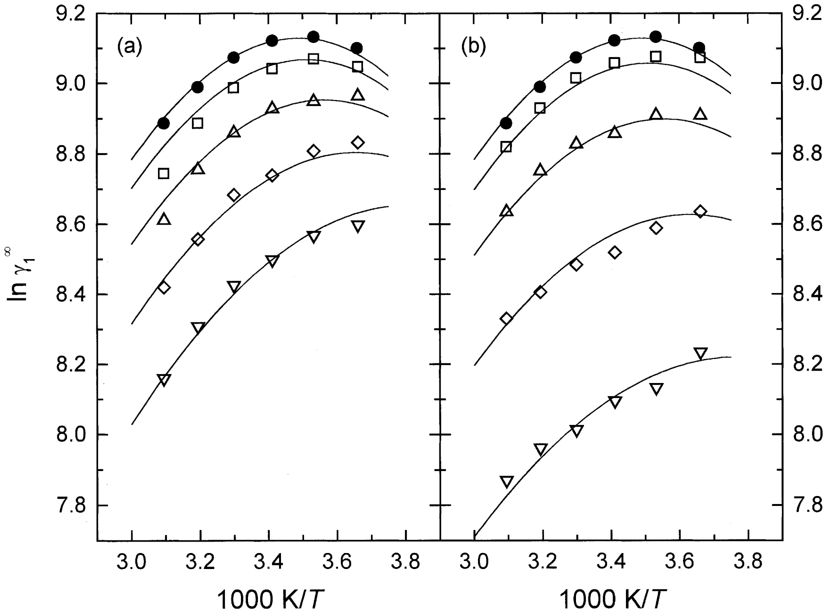

Fig. 4. The HSCE model fits (lines) to the experimental limiting activity coefficients $\ln\gamma_1^\infty$ of toluene in aqueous solutions of (a) urea and (b) guanidine as a function of temperature, at several modifier molar concentrations (mol/dm^3): ●, 0 (pure solvent water); □, 0.5; △, 1.5; ◇, 3.0; ▽, 5.0. Lines were obtained using Eq. (24). These simultaneous fits produced the parameters reported in Table 3. The experimental data are from [16].

other. According to the HSCE model, the temperature dependence of $\Delta_{sol}G_1^\infty/RT$ and the presence of its maximum (minimum in solubility) in Fig. 6b are due to the structural alteration of the water molecules in the first solvation shell around the solute. As the temperature decreases, it is the increasing importance of ΔG^{alt} that makes possible the dissolution of larger quantities of solute into water, i.e. the existence of the solubility minimum.

Table 3
The HSCE model interaction parameters, in J·mol^{-1}, fitted to ternary non-polar solute (1) +water (2) +protein denaturant (3) data, using Eq. (24)

Solute (1)	water (2) + urea (3), $\Delta_{23} = -600$		water (2) + guanidine (3), $\Delta_{23} = -1970$	
	Δ_{13}	$\sigma_{13}-\sigma_{12}$	Δ_{13}	$\sigma_{13}-\sigma_{12}$
Alkylbenzenes^a		-1480		-1760
Benzene	6270		4480	
Toluene	6012		4163	
	Δ_{13}–Δ_{12}^{c}	$\sigma_{13}-\sigma_{12}$	Δ_{13}–Δ_{12}^{c}	$\sigma_{13}-\sigma_{12}$
Alkanes^b	$-11\,722+4719/Q_1$	-1225	$-12\,878+5135/Q_1$	-2614

[a] Fitted to γ_1^∞ from [16], for urea $s_{rel} = 2.0$ % and for guanidine $s_{rel} = 2.3$ %.
[b] Fitted to γ_1^∞ (in mixed solvent)/γ_1^∞ (in water) from [15], for urea $s_{rel} = 2.8$ % and for guanidine $s_{rel} = 3.6$%.
[c] The indicated dependence on solute size was considered in the fitting.

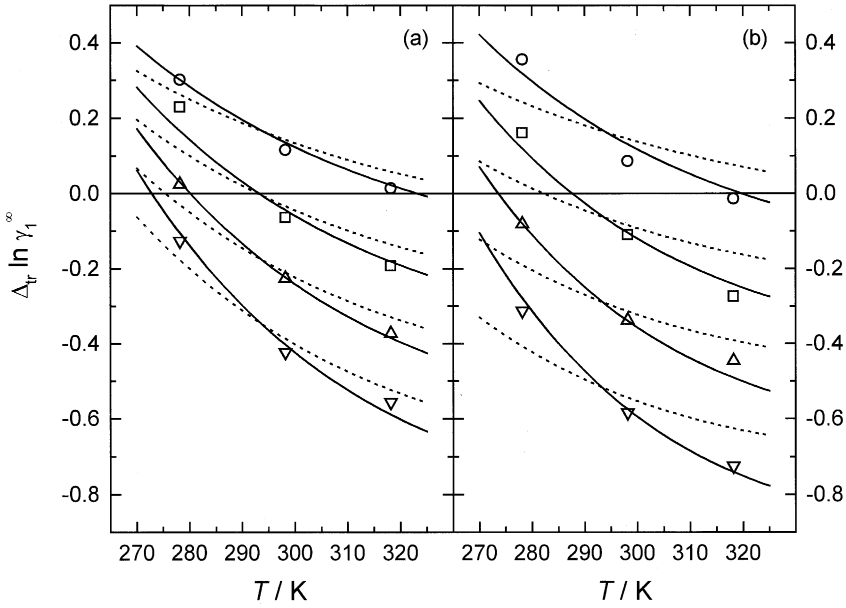

Fig. 5. The HSCE model fits to the experimental transfer limiting activity coefficients $\Delta_{tr}\ln\gamma_1^{\infty}$ of lower alkanes (methane ○, ethane □, propane △ and butane ▽) as a function of temperature. $\Delta_{tr}\ln\gamma_1^{\infty}$ refers to solute transfer from pure solvent water to an aqueous solution of (a) urea (7.0 mol/dm^3) and (b) guanidine (4.9 mol/dm^3). Full lines were obtained using Eq. (24) and dotted lines using Eq. (8). These simultaneous fits produced the parameters reported in Table 3 [in the case of Eq. (24)]. The experimental data are from [15].

3.2.2. Non-polar solutes in aqueous solutions of protein denaturants

The solute–denaturant interchange energy Δ_{13} values are positive for alkylbenzenes (in Table 3) and alkanes (inferred from $\Delta_{13} - \Delta_{12}$ in Table 3 and Δ_{12} in Table 2) indicating a dislike between these molecules. This dislike is, however, much less pronounced than that between the solute and water ($\Delta_{13} \ll \Delta_{12}$), i.e. the non-polar solute prefers to be in contact with the denaturant rather than with water. The Δ_{13} values are bigger for urea than for guanidine, this difference being more pronounced for alkylbenzenes than for alkanes. This energetic preference of the solutes by guanidine is manifested, as seen in Figs. 3–5, in a bigger solubilization of the non-polar solutes in this denaturant, as compared to urea. It is also consistent with the experimental observation that less guanidine than urea is needed to unfold a protein. For the case of alkylbenzenes, it is probable that the solute–guanidine interaction takes place not only as depicted in Fig. 1 but also between the positive charge in the guanidinium cation and the aromatic ring of benzene or toluene. This is an interaction that is not explicitly included in the HSCE model, but is reflected in the Δ_{13} values evaluated from the experimental data. The positive Δ_{13} values in Table 3 do not imply the existence of a preferential binding or a specific interaction leading to a distinct chemical entity (a solute–denaturant complex). The use of those terms is then not justified to describe the solute–denaturant interaction. This is in agreement with the conclusions reached by Loh et al. [41] from calorimetric data for sparingly soluble solutes in aqueous solutions of urea. According to our results, the non-polar solute–denaturant interaction is better described by the term 'preferential solvation of the solute by the denaturant' as discussed below.

For both alkylbenzenes and alkanes, the $\sigma_{13} - \sigma_{12}$ parameter in Table 3 is negative, clearly indicating a preferential solvation of the solute by the denaturant. This language attempts to describe

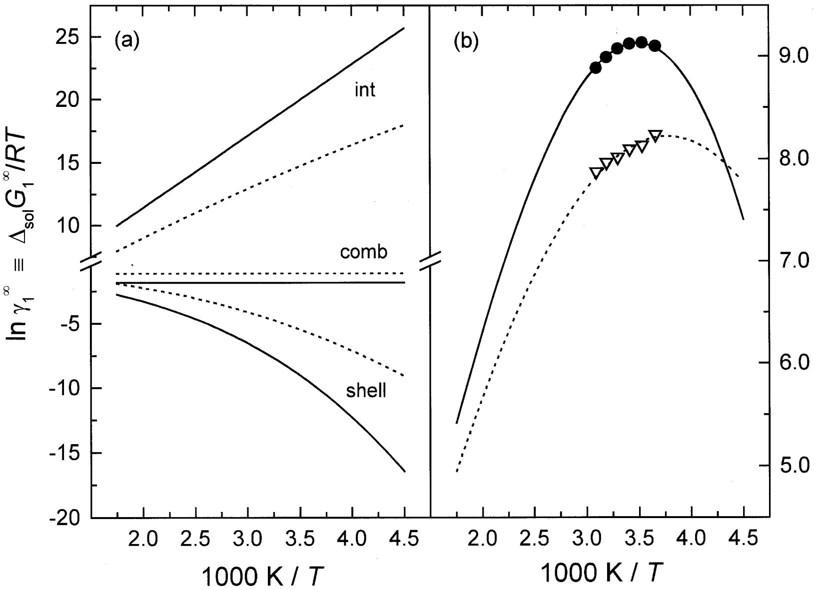

Fig. 6. The temperature dependence of the HSCE model contributions to the limiting activity coefficients $\ln\gamma_1^\infty$ of toluene in water (●) and in an aqueous solution (5.0 mol/dm^3) of guanidine (▽). In (a) the combinatorial (comb), interactional (int) and water alteration (shell) contributions were calculated using Eq. (4), Eq. (11) and Eq. (23), respectively, with the parameters in Table 2 and Table 3. Full lines are for the pure solvent water case and dotted lines for the (water + guanidine) mixed solvent case. The experimental data in (b) are from [16].

the following situation: there is a non-random distribution of water and denaturant molecules surrounding the solute, a distribution where denaturant molecules are preferentially in contact with the solute since the solute–denaturant interactions are energetically favored over the solute–water ones $|\sigma_{13}| > |\sigma_{12}|$. The $\sigma_{13} - \sigma_{12}$ parameter is more negative for guanidine than for urea implying a more pronounced preferential solvation in the case of guanidine and in agreement with the Δ_{13} values discussed above. From the Δ_{12} values in Table 2 and the Δ_{13} and $\sigma_{13} - \sigma_{12}$ values in Table 3 for benzene and toluene, it is possible to calculate $\sigma_{22} - \sigma_{33}$, its value being between -17 and -20 kJ/mol. Hence, the cohesion between water molecules is much larger than that between denaturant molecules and it remains to be the dominant cause of the hydrophobic behavior of the non-polar solutes. The presence of the denaturant only partially attenuates the hydrophobic effect.

The water–denaturant interchange energies Δ_{23} in Table 3 are negative for both urea and guanidine, indicating a favorable interaction. For binary mixtures (water + denaturant), negative Δ_{23} values imply negative deviations from Raoult's law, a fact that contradicts the experimental data for water + urea [12] and water + guanidine [13,14], the latter exhibiting larger positive deviations. However, the Δ_{23} values are relatively small so that this imperfection of the model is not significant; in fact, when Eq. (8) is used, Δ_{23} for both urea and guanidine are positive. Also, since there is a rather weak sensitivity of $\Delta_{sol}G_1^\infty/RT$ values to the Δ_{23} parameter, Δ_{23} values have a larger uncertainty than the other model parameters. The possible sources for the disagreement between the Δ_{23} values in Table 3 and the experimental observations for the water–denaturant binary mixtures are: (*i*) the fact that the binary parameter Δ_{23} was obtained here from ternary mixture data, a procedure that does not always yield reliable values; (*ii*) the simple equation to describe the mean energy of the water–denaturant interactions [Eq. (7)] is too crude and even unable to represent correctly the binary mixtures alone; and (*iii*) the fact that the model ignores the electrolyte nature of guanidine.

In a molecular simulation study [33], a negative Gibbs energy change was found for the process of approaching a benzene molecule and a urea (or guanidine) molecule both at infinite dilution in water. In the HSCE model, this process corresponds to the replacement of 1–2 and 2–3 interactions by 1–3 and 2–2 interactions. Associated with these replacements, there is a Gibbs energy change (per standard segment surface) given by $\sigma_{13} + \sigma_{22} - \sigma_{12} - \sigma_{23}$. In Table 3, $\sigma_{13} - \sigma_{12}$ is negative indicating that benzene interacts more favorably with the denaturant than with water. The difference $\sigma_{22} - \sigma_{23}$ cannot be explicitly evaluated from the thermodynamic information using the HSCE model. However, from the discussion presented above it is clear that the water–water interaction is stronger than the water–denaturant interaction and hence $\sigma_{22} - \sigma_{23}$ is also negative. Therefore, the HSCE model results are in agreement with the molecular simulation calculations.

The presence of a denaturant modifies quantitatively the three contributions to the solution Gibbs energy, as illustrated in Fig. 6a for the case of toluene in an aqueous guanidine solution. ΔG^{int} is less positive than for the pure water solvent case, owing to the relatively favorable solute–denaturant interaction. On the other hand, ΔG^{alt} becomes less negative due to the presence of the denaturant that reduces the number of water molecules in contact with the solute in other words, less water molecules undergo the alteration described by Eq. (13). The combinatorial contribution is also less negative, but its change respect to the pure water solvent case is quite small. Fig. 6b shows the total $\Delta_{sol}G_1^{\infty}/RT$ together with the experimental results. As in the pure water solvent case, the HSCE model explains these experimental results and all those in Figs. 3–5 as the result of the balance between the two (combinatorial aside) opposite sign contribu-

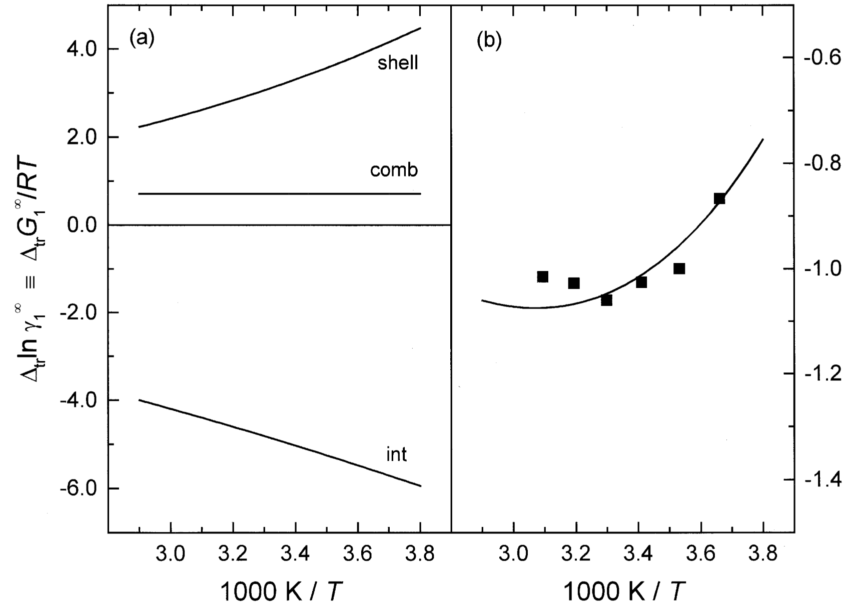

Fig. 7. The temperature dependence of the HSCE model contributions to the transfer limiting activity coefficients $\Delta_{tr}\ln\gamma_1^{\infty}$ of toluene in an aqueous solution (5.0 mol/dm^3) of guanidine (■). In: (a) the combinatorial (comb), interactional (int) and water alteration (shell) contributions were calculated using Eq. (4), Eq. (11) and Eq. (23), respectively, and used in Eq. (25) with the parameters from Table 2 and Table 3. The experimental data in (b) are from [16].

tions. The increased solubility of a non-polar solute in aqueous solutions of denaturants (as compared to that in pure water) is due to the relatively favorable solute–denaturant interaction (as compared with the very unfavorable solute–water interaction). In this sense, the presence of the denaturant decreases the hydrophobic effect. This is more clearly seen with the transfer Gibbs energy $\Delta_{tr}G_1^{\infty}$ which characterizes the process where the solute is transferred from water to aqueous denaturant solution. The $\Delta_{tr}G_1^{\infty}$ values from Eq. (25) for toluene in an aqueous guanidine solution are plotted against temperature in Fig. 7a. Here, the interactional contribution is negative, i.e. favorable, and the combinatorial and the water-alteration contributions are positive, both unfavorable to the solute transfer. As seen in Fig. 7b the net $\Delta_{tr}G_1^{\infty}$ is negative as a result of the interactional contribution overcoming the other two contributions. The results of the HSCE model in Fig. 7 provide a rationalization for the experimental $\Delta_{tr}G_1^{\infty}$ values whose relevance for the understanding of the unfolding process has been long realized [17,18].

The data in Fig. 5 possess a challenge to any model attempting to describe the solubilities of non-polar solutes in aqueous solutions of denaturants. According to Fig. 5, aqueous urea and guanidine solutions decrease the solubility of lower alkanes as compared to that in pure water, and this effect is a function of solute size and temperature. There is a threshold size at a given temperature and a threshold temperature at a given solute size for solubility enhancement. The HSCE model is useful to provide a rationalization of the data in Fig. 5. Using the parameters in Tables 1–3, the three contributions to $\Delta_{tr}G_1^{\infty}$ are plotted in Fig. 8a for the lower alkanes in urea at 298.15 K and for ethane in urea as a function of temperature in Fig. 8b. In Fig. 8a, as the alkane size is reduced the $(\Delta_{13} - \Delta_{12})$ parameter in Table 3 becomes less negative and correspondingly $\Delta_{tr}G^{int}$ is reduced in magnitude; hence, in going from butane to methane the solute–denaturant

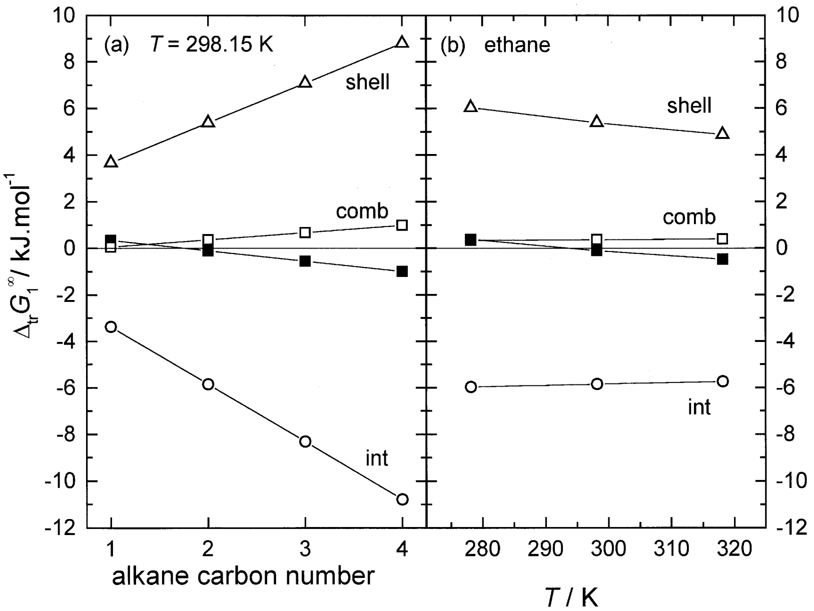

Fig. 8. The HSCE model contributions to the transfer Gibbs energy $\Delta_{tr}G_1^{\infty}$ of: (a) the lower alkanes at 298.15 K as a function of alkane carbon number; and (b) ethane as a function of temperature. $\Delta_{tr}G_1^{\infty}$ refers to solute transfer from pure solvent water to an aqueous solution of urea (7.0 mol/dm^3), the experimental data (■) being from [15]. The combinatorial (comb), interactional (int) and water alteration (shell) contributions were calculated using Eq. (4), Eq. (11) and Eq. (23), respectively, and used in Eq. (25) with the parameters from Table 2 and Table 3. Lines in (a) are only to aid visualization.

interaction is increasingly closer to the solute–water interaction. On the otherhand, the reduction in solute size decreases the number of water molecules undergoing the water alteration process and hence its contribution to the transfer Gibbs energy, $\Delta_{tr}G^{alt}$. At 298.15 K the solubility of methane in an aqueous solution of urea is lower than in pure water due to a large solute–urea dislike combined with a positive water alteration contribution that is dominant. In Fig. 8b, the temperature dependence of $\Delta_{tr}G^{alt}$ (negative) is more pronounced than that of $\Delta_{tr}G^{int}$ (positive). Then, at high temperatures $\Delta_{tr}G^{int}$ dominates enhancing ethane solubility, while at low temperatures $\Delta_{tr}G^{alt}$ is dominant, diminishing solubility.

3.3. Comparison with the modified hydration shell hydrogen bond model

In recent years, Muller has presented a modified hydration shell hydrogen bond (MHSHB) model to account for some thermodynamic properties of aqueous solutions of non-polar solutes [21,22]. This model was extended to deal with the effect on those properties due to the presence of a cosolvent (urea) [23]. Since the HSCE and MHSHB models were developed to describe the same type of systems, it is important to assess here their similarities and differences. The main similarities are that both models: (*i*) are capable to describe the data to approximately the same level of quantitative agreement; (*ii*) consider that the first solvation shell around the solute plays a crucial role in determining the thermodynamic properties of the system; and (*iii*) require a solute–size dependence for the parameters involved in the interactional contribution, in order to rationalize the lower alkane transfer Gibbs energy data. The most important difference lies, as discussed below, in the nature of the contributions to the Gibbs energy of solution or transfer. In the MHSHB model $\Delta_{sol}G_1^{\infty}$ is given by:

$$\Delta_{sol}G_1^{\infty} = \frac{3}{2}NRT(1-\xi_3)\ln\frac{K_{hs}(1+K_b)}{K_b(1+K_{hs})} + x_3(\alpha N+\beta) + N\delta_{12} \qquad (26)$$

where the equilibrium constants $K_{hs} = \exp(-\Delta H_{hs}^0/RT + \Delta S_{hs}^0/R)$ and $K_b = \exp(-\Delta H_b^0/RT + \Delta S_b^0/R)$ refer to the equilibrium between intact and broken water hydrogen bonds in the hydration shell and the bulk, respec-

Table 4
The MHSHB model parameters for alkane and alkylbenzene solutes in water and aqueous modifier solutions[a]

Solute series [b]	ΔH_{hs}^0 J·mol^{-1}	ΔS_{hs}^0 J·mol^{-1} K^{-1}	solute (1)	δ_{12} J·mol^{-1}	modifier (3)	α[e] J·mol^{-1}	β[e] J·mol^{-1}	s_{rel} %
Alkylbenzenes	10 288[c]	25.32[c]	benzene	2[c]	urea	765.8	–	3.5
					guanidine	601.1	–	3.5
			toluene	18[c]	urea	734.3	–	3.8
					guanidine	548.3	–	3.5
Alkanes	10 696[d]	27.36[d]	nC_1–nC_4	–	urea	809.5 (789)	14754 (14800)	4.2
					guanidine	333.9	24381	7.9
			nC_5,nC_6,cC_6	−42[f]				

[a] Water bulk parameters $\Delta H_b^0 = 9800$ J·mol^{-1}, $\Delta S_b^0 = 21.6$ J·mol^{-1}·K^{-1} from [21]. Modifier surface fraction, ξ_3, calculated as given in [23], $\xi_3 = 1.85x_3/(x_2 + 1.85\,x_3)$.

[b] Number of water molecules (N) surrounding each solute are: benzene, 26.7; toluene, 30.4; methane, 16.9; ethane, 21.3; propane, 24.8 and butane, 28.4 from [23].

[c] Obtained in this work. Fitted to γ_1^{∞} in pure water solvent from [16], $s_{rel} = 0.7\%$.

[d] From [21].

[e] Obtained in this work. For alkylbenzenes fitted to γ_1^{∞} from [16] and for alkanes fitted to γ_1^{∞} (in mixed solvent)/γ_1^{∞} (in water) from [15]. Values in parentheses are those from [23].

[f] Obtained in this work. Fitted to x_1^{sol} in pure water solvent from [20], $s_{rel} = 16\%$.

tively. In Eq. (26), N is the number of water-molecules surrounding the solute, x_3 is the mole fraction of denaturant and ξ_3 is, as in the HSCE model, the surface fraction of the hydration shell occupied by the denaturant. In the MHSHB model, ξ_3 is calculated as indicated in Table 4, assuming a random distribution of water and denaturant molecules around the solute. The logarithmic factor in the first term of Eq. (26) accounts for the changes, due to the presence of the solute, in the population of broken and intact hydrogen bonds. The MHSHB model assumes that the denaturant interferes little with water–hydrogen bonding and acts simply by preempting space in the solvation shell around the solute that would otherwise accommodate water molecules. The second and third terms in Eq. (26) are the interactional contributions to the Gibbs energy. The second term, whose solute size dependence is described by two adjustable parameters, α and β, is due to solute–denaturant and water–denaturant van der Waals forces. In [23] the MHSHB model was presented for the transfer Gibbs energy. In order to calculate the corresponding solution Gibbs energy, it is necessary to consider the third term in Eq. (26). This term accounts for the interaction between the solute and water molecules, characterized by δ_{12}, and cancels out when Eq. (26) is used to obtain the transfer Gibbs energy. Table 4 reports the MHSHB model parameters for the lower alkanes (methane to butane) and for benzene and toluene in pure solvent water and in aqueous solutions of urea and guanidine. The source of each parameter, either [23] or evaluated in this work, is indicated in Table 4.

Fig. 9 shows the results from the MHSHB model for benzene in a pure water solvent and in an aqueous solution of urea. Fig. 9a displays the contributions to the solution Gibbs energy according to Eq. (26), namely those due to water hydrogen bonding (first term) and due to van der Waals forces (second and third terms). Both contributions are positive and hence in the MHSHB model the experimental $\Delta_{sol}G_1^\infty/RT$ values in Fig. 9b are the result of two additive unfavorable

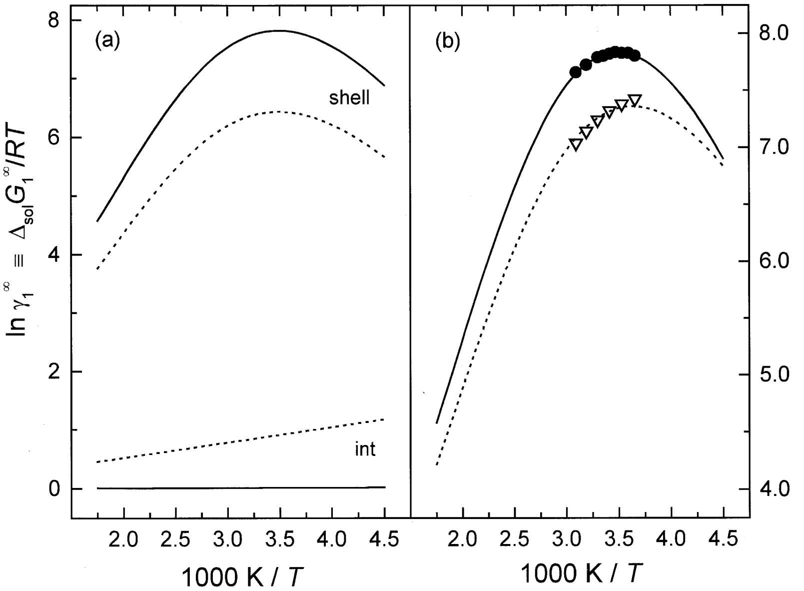

Fig. 9. Temperature dependence of the MHSHB model contributions to the limiting activity coefficients $\ln\gamma_1^\infty$ of benzene in water (●) and in an aqueous solution (5.0 mol/dm^3) of urea (▽). In (a) the interactional (int) and hydrogen bonding (shell) contributions were calculated using Eq. (26) with the parameters in Table 4. Full lines are for the pure solvent water case and dotted lines for the (water + urea) mixed solvent case. The experimental data in (b) are from [16].

contributions, in contrast with the situation in the HSCE model in Fig. 6 where a favorable contribution and an unfavorable one compete. For pure water solvent, the interactional contribution is very small while the hydrogen bonding contribution is large and accounts for practically all the experimental $\Delta_{sol}G_1^\infty/RT$ and its temperature dependence. When urea is present, Fig. 9a indicates that the hydrogen bonding contribution becomes less positive owing to the presence of less water molecules in the solvation shell around the solute. However, the interactional contribution becomes more positive. This implies that in the MHSHB model the presence of the denaturant produces a medium that interacts more unfavorably with the solute than the pure water solvent. This is not a physically plausible situation. This drawback of the MHSHB model is not a peculiarity of its application to alkylbenzenes in aqueous solutions of denaturants data, neither a consequence of our fitting of the model parameters. Instead, it reflects a general behavior of the MHSHB model. While for benzene and toluene the solute–water interaction is practically zero, for alkane solutes the solute–water interaction term in $\Delta_{sol}G_1^\infty$ is even negative, i.e. favorable (see Table 4). Again, this is difficult to accept as a realistic physical result. It appears that although the MHSHB model is able to describe quantitatively the experimental data, it is not free of inconsistencies and conceptual problems.

3.4. Other thermodynamic functions

The high accuracy of the solution Gibbs energy data reported in [16] allowed their extrapolation above the experimental temperature range (273–323 K), and the calculation of solution enthalpies and entropies with a reasonable degree of confidence. This extrapolation and calculations were performed using the correlation functions obtained from the data under the assumption of a temperature independent solution heat capacity [16]. Fig. 10a shows the experimental $\Delta_{sol}G_1^\infty$, $\Delta_{sol}H_1^\infty$ and $-T\Delta_{sol}S_1^\infty$ for benzene in pure water solvents and in an aqueous solution of urea, while Fig. 10b displays the results from the HSCE model. The comparison between the two panels

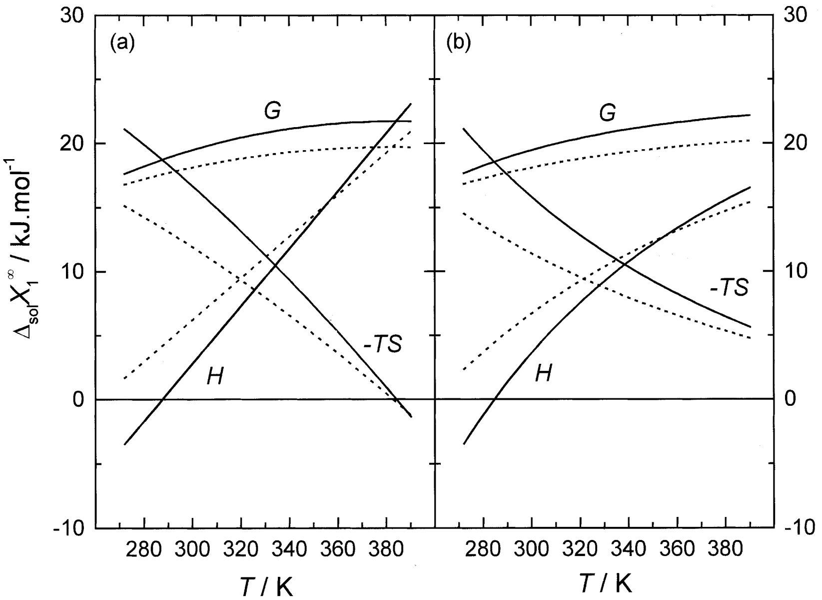

Fig. 10. Thermodynamic functions of solution at infinite dilution, $\Delta_{sol}X_1^\infty(X = G,H,-TS)$, for benzene in pure water (full lines) and in an aqueous solution (5.0 mol/dm^3) of urea (dotted lines), as a function of temperature. Curves in (a) are experimental from [16] and those in (b) are HSCE model calculations.

in Fig. 10 indicates that the HSCE model is able to reproduce the main features of the experimental results. Fig. 10 shows that within the 273–390 K temperature range, $\Delta_{sol}G_1^{\infty}$ changes by approximately 15%, but its enthalpic and entropic contributions change drastically. At low temperatures, the entropic contribution to $\Delta_{sol}G_1^{\infty}$ is dominant, while at high temperatures, $\Delta_{sol}G_1^{\infty}$ is dominated by its enthalpic contribution. In other words, the low solubility or hydrophobicity of benzene in pure water and in an aqueous solutions of urea, is due to an entropic effect at low temperatures and to an enthalpic effect at high temperatures. In this context, it is important to mention that the calculated solution heat capacities at infinite dilution, $\Delta_{sol}C_{P1}^{\infty}$, for benzene and toluene in pure water solvent show a stronger temperature dependence than experimentally observed [42]. In pure water solvent, $\Delta_{sol}C_{P1}^{\infty} = T(\partial\Delta_{sol}S_1^{\infty}/\partial T) = -T(\partial^2\Delta G^{alt}/\partial T^2)$ is only due to the structural alteration of water molecules in the first solvation shell around the solute. Hence, a possible source for the unsatisfactory $\Delta_{sol}C_{P1}^{\infty}$ temperature dependence in the HSCE model is having ignored the change in Gibbs energy involved in the spatial alignment (prior to the establishment of the interaction described by Eq. (11)) of water molecules over the solute surface, as shown in Fig. 1. Nevertheless, in a global balance between achievements and drawbacks, the HSCE model provides a consistent and meaningful scheme to interpret, at the molecular level, the experimental observations for non-polar solutes in water and in aqueous solutions of denaturants.

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Appendix A: Relationship between the HSCE model and the two-state relaxation model

In [35], it was considered that N water molecules in the first hydration shell around the solute undergo a relaxation process that was described using a two-state model. The Gibbs energy for the relaxation process ΔG^{rlx}, i.e. for the process where unrelaxed water molecules (initial state) are transformed into an equilibrium mixture of relaxed and unrelaxed water molecules (final state) is given by:

$$\Delta G^{rlx} = NRT\ln[1 + \exp((\Delta h/R)(1/T - 1/T_m))] \qquad (A.1)$$

where Δh is the energy difference between the two energy states and T_m is the temperature at which both states are equally populated. Eq. (A.1) differs from Eq. (2) in [35] since in obtaining the latter the process (relaxed water to equilibrium mixture) was wrongly considered. The passage of water molecules from the unrelaxed state to the relaxed state is characterized by an equilibrium constant that, using Eq. (1.8) in [35] for the fraction of water molecules at each energy level, is given by:

$$K = \exp((\Delta h/R)(1/T - 1/T_m)) \qquad (A.2)$$

Substitution of Eq. (A.2) into Eq. (A.1) yields Eq. (21) in the text. It appears that the structural changes occurring to water molecules in the first solvation shell around the solute are equivalently described by the water alteration reaction, Eq. (13), or by the relaxation process modeled in [35]. The relationship between the parameters in the HSCE model and those in the two-state description are easily found as:

$$\Delta H^0 = -\Delta h \qquad (A.3)$$

and

$$\ln K_{298} = (\Delta h/R)(1/298.15 - 1/T_m) \qquad (A.4)$$

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