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# Modeling Gibbs energies of solution for a non-polar solute in aqueous solutions of the protein stabilizers glycerol and ethylene glycol

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**Abstract**

The Hydration Shell Chemical Equilibrium Model (HSCE) has been applied to Gibbs energies of solution data for toluene in aqueous solutions of the protein stabilizers glycerol and ethylene glycol. The HSCE model fits the experimental data to nearly experimental uncertainty. This satisfactory rendering of the data provides certainty on the physical significance of the model parameters and allows a description, from the molecular point of view, of the behaviour of a non-polar solute in aqueous solutions of protein stabilizers. The toluene–stabilizer interchange energy is positive indicating a dislike between toluene and the stabilizer molecules. This dislike is, however, much less pronounced than that between the solute and water, i.e. the non-polar solute prefers to be in contact with the stabilizer rather than with water. The cohesion between water molecules is much larger than that between stabilizer molecules and it remains to be the dominant cause of the hydrophobic behaviour of the non-polar solute. Since the solute–stabilizer interactions are energetically favoured over the solute–water ones, in the vicinity of the solute the stabilizer molecules are preferred over water ones. However, there is no specific interaction leading to a distinct chemical entity (a solute–stabilizer complex). Thus, the non-polar solute–stabilizer interaction is better described by the term 'preferential solvation of the solute by the stabilizer'.


*Keywords:* Protein stabilization; Aqueous stabilizer solvent; Non-polar solute; Hydrophobic hydration model; Preferential solvation; Glycerol; Ethylene glycol

## 1. Introduction

A common procedure for maintaining the native structure of proteins is their storage at low temperatures in aqueous solutions of polyols and sugars [1]. The manner in which these compounds increase protein heat stability or reduce the extent of denaturation by other chemical agents is not well established. The complexity of protein stabilization mechanism makes it necessary to dissect this multifaceted phenomenon into simple components, so that some of its aspects can be better

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understood. Within this context, we study in this work the molecular interactions occurring between a simple non-polar substance and protein stabilizers in aqueous media.

Upon stabilization, the non-polar chemical groups in the protein interior are prevented from being exposed to the aqueous media. Hence, thermodynamic studies involving amino acids [2] or other small and sparingly soluble non-polar solutes in water and in (water + stabilizer) solutions are of importance. Recently, we have reported experimental infinite dilution activity coefficients $\gamma_1^{\infty}$ in the 273–323 K temperature interval for toluene in (water + glycerol) solutions at four stabilizer concentrations (0.5–5.0 mol $dm^{-3}$), in (water + ethylene glycol), (water + glucose) and (water + sucrose) solutions at 1.5 mol $dm^{-3}$ and in a (water + trehalose) solution at 0.5 mol $dm^{-3}$ [3]. 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, at infinite dilution, from the pure liquid state to the aqueous solution. The data were analyzed using a classical thermodynamic scheme, which allowed the derivation of the enthalpies, entropies and heat capacities, and a detailed discussion at the macroscopic level of the several observed trends [3]. Similar $\gamma_1^{\infty}$ data were reported for benzene and toluene in two protein denaturants, urea and guanidine hydrochloride [4]. In this case, apart from the classical thermodynamic treatment, the data were also analyzed in detail using a simple molecular model which we termed the Hydration Shell Chemical Equilibrium Model (HSCE) [5]. One of the main conclusions was that the solute–denaturant interaction is not specific, i.e. leading to a distinct chemical entity, but rather a preferential solvation of the solute by the denaturant was found. In this work, we study in detail the toluene–glycerol and toluene–ethylene glycol interactions using the HSCE model. The main goals are to compare these interactions with those occurring between the same solute and protein denaturants and also to establish whether the data are consistent with preferential solvation or with the formation of a solute–stabilizer complex.

## 2. Brief description of the Hydration Shell Chemical Equilibrium Model

A complete and detailed description of the HSCE model is given in Ref. [5]; here, we only highlight its main characteristics. The HSCE model was developed to describe 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) that can be, for example, a protein stabilizer or a protein denaturant. The model considers $\Delta_{sol}G_1^{\infty}$ being composed of three contributions which originate from combinatorial entropy effects, molecular interactions and the structural alteration of water molecules in the first solvation shell around the solute. All contributions depend on the molecular geometry of the species involved. For the combinatorial contribution, the Flory–Huggins expression was employed:

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

where $x_2$ and $x_3$ are the mole fractions of water and modifier, respectively, and the $R_i$ the ratios of the molecules van der Waals volumes to a standard segment volume. $\Delta G^{comb}$ is a negative contribution that increases in absolute magnitude with increasing solute size and decreases with increasing modifier concentration.

The interactional contribution results from the balance of the pair potential energies involved in three hypothetical steps leading to the positioning of a non-polar molecule in the aqueous solution, namely: (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. The total interactional contribution is:

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

where $\xi_3$ is the fraction of the cavity surface occupied by the modifier, $Q_1$ is the ratio of the

solute area to that of a standard segment and $\Delta_{ij}$ are the interchange energies defined as $\Delta_{ij} \equiv \sigma_{ij} - 1/2 \times (\sigma_{ii} + \sigma_{jj})$ with $\sigma_{ij}$ being 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. Since for hydrophobic solutes $\Delta_{12}$ is dominant, $\Delta G^{\text{int}}$ is a large positive contribution.

Alteration of the liquid water molecular structure due to the presence of the solute was described employing a chemical equilibrium approach, without virtually specifying neither a concrete mechanism for this alteration, nor a particular spatial arrangement of water molecules in both initial and final states. This contribution is:

$$\Delta G^{\text{alt}} = -(Q_1/\overline{Q_2})\xi_2 RT \ln(1+K) \qquad (3)$$

where $\xi_2$ is the fraction of the cavity surface occupied by water, $\overline{Q_2}$ is the portion of the cavity taken by one water molecule and $K$ is the equilibrium constant for the chemical equilibrium between normal and altered water. The temperature dependence of $K$ is given by $\ln K = \ln K_{298} + (\Delta H^0/R)\ (1/298.15 - 1/T)$ with $\Delta H^0$ being the temperature independent standard enthalpy change for the alteration. $\Delta G^{\text{alt}}$ is proportional to the solute contact area and is always negative. According to Eq. (3), water molecules in direct contact with the solute accommodate their own structure to decrease the total free energy of the system. Hence, in the HCSE model the Gibbs energy of solution is the result of the balance between contributions of different signs.

In obtaining Eqs. (1)–(3), it was assumed that the composition of the mixed solvent in the first solvation shell around the solute is the same as in the bulk, i.e. a random mixing occurs in direct contact with the solute. In this case, the local surface fractions $\xi_i$ are given by:

$$\xi_2 = \frac{x_2\overline{Q_2}}{x_2\overline{Q_2} + x_3\overline{Q_3}}; \qquad \xi_3 = 1 - \xi_2 \qquad (4)$$

In order to account for preferential solvation, the local composition concept of Wilson [5] can be used. Accordingly, $\xi_2$ can be expressed as:

$$\xi_2 = \frac{x_2\overline{Q_2}}{x_2\overline{Q_2} + x_3\overline{Q_3}\exp[-(\sigma_{13} - \sigma_{12})/RT]} \qquad (5)$$

where a non-zero $\sigma_{13} - \sigma_{12}$ value indicates non-randomness and its sign indicates the component of the mixed solvent that preferentially solvates the solute.

## 3. Results and discussion

The HSCE model involves six adjustable parameters if Eq. (5) is used; alternatively, if Eq. (4) is employed, there are five parameters. These parameters can be evaluated from the experimental Gibbs energies of solution. Three or four of these parameters ($\Delta_{12}$, $\Delta_{13}$, $\Delta_{23}$ and, if Eq. (5) is used, $\sigma_{13} - \sigma_{12}$) characterize pair interactions and the remaining two parameters ($\ln K_{298}$ and $\Delta H^{\circ}$) describe the structural alteration of water in the first solvation shell. For the particular case of a hydrophobic solute in pure solvent water ($x_3 = 0$), the HSCE model equations above reduce to contain three parameters. These parameters have been evaluated in Ref. [5] using toluene + water data and their values are: $\Delta_{12} = 15980$ J mol$^{-1}$, $\ln K_{298} = -1.0434$ and $\Delta H^{\circ} = -6214$ J mol$^{-1}$. In order to fit the remaining three or two parameters ($\Delta_{13}$, $\Delta_{23}$ and $\sigma_{13} - \sigma_{12}$) to $\gamma_1^{\infty}$ data for toluene in (stabilizer + water) solutions, it is necessary to first assign the $R$ and $Q$ values for the stabilizers. The $R$'s were evaluated as described in Ref. [5], their values being 4.7897 and 3.3448 for glycerol and ethylene glycol, respectively. The $R$ values for toluene and water are given in Ref. [5] and are 3.9228 and 0.9200, respectively. In the previous application of the HSCE model to $\gamma_1^{\infty}$ data for benzene and toluene in aqueous solutions of the protein denaturants urea and guanidine hydrochloride [5], the $\overline{Q}$ values were obtained considering that water and each denaturant interact with the non-polar hydrocarbon solute through their hydrogen atoms facing the hydrocarbon surface. For urea and guanidine hydrochloride, this interaction was considered to occur with two of their hydrogen atoms, while for water the involvement being just for one of its hydrogen atoms. These spatial orientations are supported by quantum mechanical ab initio gas-phase calculations and molecular

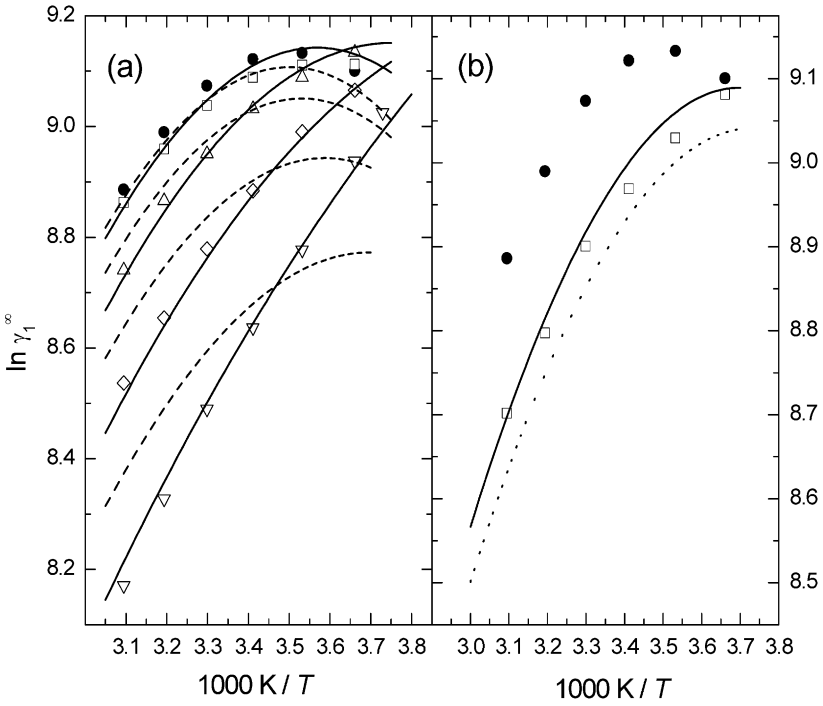


Fig. 1. HSCE model fits to the experimental limiting activity coefficients, $\ln\gamma_1^\infty$, of toluene in (a) aqueous solutions of glycerol as a function of temperature, at several modifier molar concentrations (mol dm$^{-3}$): ●, 0 (pure solvent water); □, 0.5; △, 1.5; ◇, 3.0; ▽, 5.0. Full lines were obtained using Eq. (5) and dashed lines using Eq. (4). The simultaneous fits using Eq. (5) produced the parameters reported in Table 1 and (b) an aqueous solution of ethylene glycol as a function of temperature, at a unique modifier molar concentration (mol dm$^{-3}$): ●, 0 (pure solvent water); □, 1.5. The dotted line is a prediction calculated using the parameters obtained for glycerol simultaneous fits. The full line was obtained fitting only the $\sigma_{13}-\sigma_{12}$ parameter, whose value is reported in Table 1. All the experimental data are from [3].

simulation studies [6]. For glycerol and ethylene glycol, to our knowledge there are no similar studies providing information about the spatial orientation of these compounds when they interact with an aromatic ring. Hence, in this work we have assumed that these protein stabilizers interact with toluene in an orientation such that two of their hydroxyl groups are facing the hydrocarbon surface. In our calculations we used a value of 0.12 nm for the van der Waals radius of the hydrogen atom, and the inter-atomic distances and angles reported for the protein stabilizers from X-ray diffraction studies [7]. The obtained $\overline{Q}$ values are 0.305 and 0.296 for glycerol and ethylene glycol, respectively; for water the $\overline{Q}$ value from Ref. [5] is 0.109.

Table 1
HSCE model interaction parameters, in J mol$^{-1}$, fitted to ternary toluene (1) + water (2) + protein stabilizer (3) data, using Eq. (5)

| | Glycerol | Ethylene glycol |
|---|---|---|
| $\Delta_{13}$ | 6224 | 6224 |
| $\Delta_{23}$ | −1713 | −1713 |
| $\sigma_{13}-\sigma_{12}$ | −3266 | −2962 |

The HSCE model parameters describing the toluene–glycerol and water–glycerol interactions were evaluated employing the experimental temperature and glycerol concentration dependences of the Gibbs energies of solution [3]. The performances of Eqs. (4) and (5) are shown in Fig. 1a, the parameters corresponding to the employment of Eq. (5) being reported in Table 1. The standard deviations of the fit are lower for the case of Eq.

(5) by a factor of 5, the difference between these standard deviations being statistically significant; also, the parameter $\sigma_{13}-\sigma_{12}$ is different from zero with statistical significance. It appears then that consideration of preferential solvation, i.e. Eq. (5), gives a better rendering of the experimental data. In Ref. [3], the Gibbs energies of solution for toluene in aqueous solutions of other four protein stabilizers (ethylene glycol, glucose, sucrose and trehalose) were also reported. For each of these four stabilizers, the data are available at six temperatures but at a single stabilizer concentration. Fitting the two or three HSCE model parameters to data gathered at a unique stabilizer concentration is not justified. However, it is known that stemming from the close chemical similarity between glycerol and ethylene glycol, both these substances can be represented in the group contribution estimation methods by using the same generic interactional groups [8]. This implies that the HSCE model should be able to predict the solution Gibbs energy data for toluene in (water + ethylene glycol) solutions using the parameters obtained for glycerol. This calculation is shown in Fig. 1b where it is readily seen that the HSCE model predicts very well the ethylene glycol data. This description can be further improved by fitting the $\sigma_{13}-\sigma_{12}$ parameter, as the only adjustable parameter. This is also displayed in Fig. 1b, the resulting value for the $\sigma_{13}-\sigma_{12}$ parameter being in Table 1.

Fig. 1 shows that, despite its simplicity, the HSCE model fits the experimental data to nearly experimental uncertainty. At the correlation level, it is able to represent the data with fewer parameters than those needed using a classical thermodynamic scheme [3]. At the interpretation level, it is a useful tool to explain, from the molecular point of view, the behaviour of non-polar solutes in aqueous solutions of modifiers. This stems from the physical significance of the model parameters and their plausible values that we now discuss. The solute–stabilizer interchange energy $\Delta_{13}$ value in Table 1 is positive indicating a dislike between toluene and the stabilizer 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 stabilizer rather than with water. This energetic preference of toluene for glycerol or ethylene glycol is manifested, as seen in Fig. 1, in a bigger solubilization of the non-polar solute in the aqueous solutions of these stabilizers, as compared to pure water. In this respect, the protein stabilizers studied in this work act is the same manner as the protein denaturants, urea and guanidine hydrochloride [4,5]. Note, however, that in Fig. 1a the glycerol concentrations (0.5 and 1.5 mol $dm^{-3}$) and temperatures (273.15 K and its immediate vicinity) where less of the non-polar solute can be solubilized are those often used to store proteins, to prevent them from denaturing. As seen in Fig. 1a, at these stabilizer concentrations and temperatures the HSCE model gives a satisfactory rendering of the reduction of non-polar solubility in aqueous glycerol solutions, as compared with the pure water solvent case.

The positive $\Delta_{13}$ value in Table 1 does not imply the existence of a preferential binding or a specific interaction leading to a distinct chemical entity (a solute–stabilizer complex). This is in agreement with the conclusions reached when proteins were denaturated with guanidine hydrochloride in the presence of glycerol [1]. Thus, the non-polar solute–stabilizer interaction is better described by the term 'preferential solvation of the solute by the stabilizer'. The $\sigma_{13}-\sigma_{12}$ parameter for glycerol and ethylene glycol are negative and small, clearly indicating a preferential solvation of the solute by the stabilizer. This means that there is a non-random distribution of water and stabilizer molecules surrounding the solute; stabilizer molecules are preferentially in the vicinity of the solute since the solute–modifier interactions are energetically favoured over the solute–water ones ($|\sigma_{13}| > |\sigma_{12}|$). From the $\Delta_{12}$ value given above and the $\Delta_{13}$ and $\sigma_{13}-\sigma_{12}$ values in Table 1, it is possible to calculate $\sigma_{22}-\sigma_{33}$, its value being between $-13$ and $-14$ kJ/mol. Hence, the cohesion between water molecules is much larger than that between glycerol (or ethylene glycol) molecules and it remains to be the dominant cause of the hydrophobic behaviour of the non-polar solute. The presence of the stabilizer only partially attenuates the hydrophobic effect. Finally, the water–modifier interchange energy $\Delta_{23}$ in Table 1 is negative, indicating a favourable water–stabilizer interac-

tion. For binary mixtures, negative $\Delta_{23}$ values imply negative deviations from Raoult's law, a fact that is in agreement with the experimental data for water + glycerol [9].

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