

Size Dependence of Solvation Gibbs Energies: A Critique and a Rebuttal of Some Recent Publications

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The definition of the solvation Gibbs energy of a solute A in any solvent is repeated in exactly the same way as was done almost 20 years ago. Some recent publications that challenged this definition and suggestion “correction” terms, are critically examined. It is shown that these newly suggested quantities are irrelevant to the solvation process.

1. Introduction

The question of how best to capture the qualitative idea of *solvation*, i.e., the interaction of one molecule (usually referred to as a solute) with its environment (usually referred to as a solvent), is a fundamental one in solution chemistry and recently also in biochemistry.

Over the past 20 years, one of us has been recommending the use of a certain measure of the *solvation Gibbs energy* (SGE) of a solute at an arbitrary concentration in a solvent. Twenty years ago there were several, different “standard states” that were used to measure solvation within the realm of thermodynamics. It has been shown that by introducing the concept of the pseudo chemical potential (PCP), a better and a more general measure of solvation Gibbs energy could be achieved. This new measure of SGE and its relative advantages over older ones have been discussed in detail in two recent monographs.^{1,2}

In two papers published in 1991, Sharp et al.^{3,4} have challenged the validity and generality of this new measure of SGE. They stated that “solvation free energies that have been extracted from such experimental data [i.e., partition coefficients] have been evaluated incorrectly” and added that “This expression [i.e., $\Delta G = -RT \ln K$, where K is the partition coefficient] is correct only under special circumstances”. (Remarks in square brackets have been added by the present authors). They explained that if “the solute and the solvent molecules are of different sizes, an additional term enters into the analysis. This leads to much larger transfer free energies”.

In 1993 Ben-Naim and Mazo published a criticism of these papers and showed that both the procedure of deriving and the conclusion derived from the “corrected” quantities are incorrect. Similar criticisms were published by Holtzer.^{6,7}

Further contributions to this subject are contained in a special issue of *Biophysical Chemistry* 1994, 51 devoted to “Thermodynamics of Hydration” and edited by A. A. Rashin. In particular, papers by Ben-Naim⁸ and Sitkoff et al.⁹ are pertinent to the present discussion. These are also accompanied by written discussions by other authors, that are valuable in exposing the sharp differences in points of view. More recently, four new articles have appeared that seem to be supporting the Sharp et al. views. The first two were by Tuñon et al.,^{10,11} who derive a quantity that does have the form advocated by Sharp

et al.⁴ They used this quantity to estimate the water–hydrocarbon surface tension. Having succeeded in obtaining almost perfect agreement with the experimental quantity, they claimed that their quantity is indeed useful. The other two papers^{12,13} contain a new discussion of the applicability of the Flory–Huggins theory to the problem of solvation. Referring to our and Holtzer’s criticism in refs 5–8, Kumar et al.¹² concluded that “The sweeping criticisms of the applicability of the FH theory to partition experiments that have appeared in recent literature are therefore not valid”.

Although we felt that the controversy had been settled after publishing our paper,⁵ we feel now compelled to write again on the same subject, first, to clarify that our criticism is still valid, and second, to examine the question of the usefulness of the new quantities that are claimed to convey information on SGE. Finally, we would like to convey a word of warning to chemists and biochemists who use the new “corrected” quantities in their research of biochemical processes, having been impressed by the fact that now there exists an *exact* derivation of these new quantities.

In section 2 we review of the definitions of the process of solvation, the PCP and the SGE. This is followed by two applications of the SGE: for solubility and for estimating the equilibrium constant for a chemical reaction in a liquid phase. Although these applications are well-known, we feel it is important to expose these applications and to contrast them with some dubious applications that have recently appeared in the literature. We refer specifically to the evaluation of surface tensions from SGEs. The latter have been used to justify the applicability of the newly “corrected” quantity of SGE. In section 3 we discuss the work of Tuñon et al. and of the Sharp–Honig group’s more recent publications. We conclude in section 4 with some words of warning addressed to solution chemists and to biochemists.

2. Definition and Applications of the SGE

Our starting point is the general expression for the chemical potential of any solute α in any phase l :^{1,2}

$$\mu_{\alpha}^l = \mu_{\alpha}^{*l} + kT \ln \rho_{\alpha}^l \Lambda_{\alpha}^3 \quad (2.1)$$

where ρ_{α}^l is the number density N_{α}/V of α in l and Λ_{α}^3 is the momentum partition function or the thermal de Broglie wavelength. μ_{α}^{*l} is the PCP of α in l .

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In general, μ_α^{*l} is a complicated composition dependent quantity, not appreciably simpler than μ_α itself in nonideal solutions. What makes the PCP a useful and interesting quantity is its statistical mechanical interpretation. It is easy to show that μ_α^{*l} represents the change in Gibbs energy of a solution when one molecule of type α is inserted at a fixed position (i.e., no translation) in l at constant T and P and the solution composition.^{1,2} This identification is valid within the confines of a classical statistical mechanical treatment of the system. Otherwise, it is very general and applies for any solute (of any size and shape) in any solvent, of any composition (including pure liquid consisting of α molecules^{1,2}). One cannot measure μ_α^{*l} directly. On the other hand, differences in μ_α^{*l} can be measured. Consider two phases l_1 and l_2 at equilibrium. From the condition of equilibrium

$$\mu_\alpha^{l_1} = \mu_\alpha^{l_2} \quad (2.2)$$

It follows that

$$\Delta G_\alpha^*(l_1 \rightarrow l_2) = \mu_\alpha^{*l_2} - \mu_\alpha^{*l_1} = -RT \ln (\rho_\alpha^{l_2}/\rho_\alpha^{l_1})_{\text{eq}} \quad (2.3)$$

where R is the gas constant, normally used instead of k , in thermodynamic treatment of solvation. In (2.3), ΔG_α^* is the difference in the PCP of α in the two phases l_1 and l_2 , and corresponds to the *change* in the Gibbs energy for the process of transferring α from a fixed point in l_1 to a fixed point in l_2 . The last equality on the right-hand side (rhs) of (2.3) provides a means of measuring ΔG_α^* simply by measuring the density ratio $\rho_\alpha^{l_2}/\rho_\alpha^{l_1}$ at equilibrium.

In particular, if one of the phases, say l_1 , is an ideal-gas phase, then ΔG_α^* (i.g. $\rightarrow l$) will be referred to as the solvation Gibbs energy of α in the phase l . As we shall see below, in the application of SGE for calculating equilibrium constants, one can choose any fixed reference system, say pure hexane. Clearly, since no solute-solvent interactions are involved in the ideal-gas phase, the choice of the ideal gas as a reference system is the simplest conceptually. For this choice ΔG_α^* (i.g. $\rightarrow l$) conveys the totality of interactions of α with all molecules of l . Therefore ΔG_α^* (i.g. $\rightarrow l$) is the best quantity that measures the SGE of α . We shall see in the next section that another choice of an ideal-gas phase cannot be used as a reference system, since that phase depends on the properties of l . There are two other quantities that are closely related to the PCP but are different from it. One is the standard chemical potential μ_α^o of α defined by

$$\mu_\alpha^o = \lim_{\rho_\alpha \rightarrow 0} (\mu_\alpha - RT \ln \rho_\alpha) \quad (2.4)$$

μ_α^o is independent of ρ_α and is a function of T and P and the *solvent* composition. The relation between μ_α^{*l} and μ_α^o is

$$\mu_\alpha^o = RT \ln \Lambda_\alpha^3 + \lim_{\rho_\alpha \rightarrow 0} \mu_\alpha^{*l} \quad (2.5)$$

Clearly μ_α^o applies only for very dilute solutions of α in the liquid phase l , whereas μ_α^{*l} can be applied in a solution of any concentration of α including pure α . Clearly, in the ideal-dilute range μ_α^{*l} is only trivially different from μ_α^o , and $\Delta G_\alpha^*(l_1 \rightarrow l_2)$ is exactly equal to $\Delta G_\alpha^o(l_1 \rightarrow l_2)$, when α is dilute in both l_1 and l_2 . In this regime the PCP does not add anything quantitatively new to the long established custom of using standard SGEs. It does, however, add a new conceptual feature. It identifies the standard SGE with that of a definite physical process of immersing a solute α into a fixed position in the

fluid. The use of the PCP goes beyond this, however. It extends the ability to make a quantitative measure of SGE from very dilute to any arbitrary concentration. Thus, in using the *standard* SGE, one can talk of the solvation of one water molecule in pure alcohol solution or of one alcohol molecule in pure water. In contrast, the SGE as defined here can be applied to any mixture of water and alcohol. This infinitely increases the range of applicability of the concept of solvation.

The second quantity, sometimes confused with the PCP, is the *excess* chemical potential μ_α^{EX} . This quantity was originally used in the context of deviation from *symmetrical* ideal solution (for more details see ref 2, chapter 6). More recently the concept of excess chemical potential was also defined as the difference between the actual chemical potential of α and that of the same species α in an ideal-gas phase at the same concentration. Thus,

$$\mu_\alpha^{\text{EX}} = \mu_\alpha^l - \mu_\alpha^{\text{ig}} = \mu_\alpha^{*l} + RT \ln \rho_\alpha^l \Lambda_\alpha^3 - [RT \ln \rho_\alpha^{\text{ig}} \Lambda_\alpha^3 q_\alpha^{-1}] \quad (2.6)$$

If we choose $\rho_\alpha^l = \rho_\alpha^{\text{ig}}$, we have the relation

$$\mu_\alpha^{\text{EX}} = \mu_\alpha^{*l} + RT \ln q_\alpha \quad (2.7)$$

where q_α is the internal partition function of an α molecule.

The two quantities become identical for structureless particles, for which $q_\alpha = 1$. We therefore urge that the term *excess* not be applied to the PCP. To do so only contributes to confusion.

We now turn to discuss two applications of the SGE. The simplest and most straightforward one is the calculation of the partition coefficient (or relative solubility in two phases). This follows directly from 2.3. We stress here that the partition coefficient of α between two phases l_1 and l_2 can be obtained from ΔG_α^* for any concentration of α in l_1 and l_2 at equilibrium. In the particular case when α is dilute in both l_1 and l_2 , the standard Gibbs energy can also be applied. It is however incorrect to apply the “corrected” SGE as suggested by Sharp et al. or by Tuñón et al. (see next section) to estimate partition coefficients or solubilities.

The second, perhaps more important, application of the SGE is for calculating the ratio of the equilibrium constants of any conversion between two states of a biomolecule, in an ideal gas and in a liquid phase. This is particularly important for biochemical processes, where the solvent is believed to play a dominant role in determining equilibrium constants. As an example, consider the conversion between conformers, say A and B of a macromolecule



for which the equilibrium constant, in an ideal-gas phase, is

$$K^{\text{ig}} = (\rho_B/\rho_A)_{\text{eq}} = q_B/q_A \quad (2.9)$$

where q_A and q_B are the partition functions of a single A and B molecules, respectively.

When the same process takes place in a liquid phase l , (2.9) is modified into

$$K^l = (\rho_B/\rho_A)_{\text{eq}} = \exp[(\mu_B^{*l} - \mu_A^{*l})/kT] = (q_B/q_A) \exp[(\Delta G_B^* - \Delta G_A^*)/kT] \quad (2.10)$$

We see that, in order to calculate the ratio of the equilibrium constants K^l/K^{ig} , one needs the difference in the SGE of A and B in the liquid phase l . Note also that if both A and B are very

dilute in l , the conventional standard SGE can also be used instead of the SGEs in (2.10).

We stress again that the “corrected” quantities suggested either by Sharp et al.,⁴ or by Tuñón et al.^{10,11} cannot be used for this purpose. The usage of such quantities will introduce additional factors in (2.10) that are irrelevant to solvation, hence, to the equilibrium constant as well.

3. Critique of the newly Proposed Definitions of Solvation Gibbs Energy

Regarding the initially suggested “correction” to the SGE by Sharp et al.,³ we have already shown that that “correction” is inadequate. There is no need to repeat the arguments here; our earlier “sweeping criticism”⁵ is still valid. In this section we address ourselves to some more recent publications that have appeared *after* the publication of our rebuttal paper.⁵

Tuñón et al. claim that they have derived an exact expression of the type suggested by Sharp et al. They start by defining the quantity¹⁰

$$G^* = N_A \mu_A^* + N_B \mu_B^* \quad (3.1)$$

where μ_B^* and μ_A^* are the PCP of A and B, respectively. By differentiating with respect to N_A they obtain

$$\mu_A^r = \left(\frac{\partial G^*}{\partial N_A} \right)_{T,P,N_B} = \mu_A^* + N_A \left(\frac{\partial \mu_A^*}{\partial N_A} \right) + N_B \left(\frac{\partial \mu_B^*}{\partial N_A} \right) = \mu_A^* + kT(r - 1) \quad (3.2)$$

where $r = \bar{V}_A/\bar{V}_B$ is the ratio of the partial molar volumes of A and B.

The first and second equalities on the rhs of (3.2) are exact expressions. Tuñón et al. commented that μ_A^r includes our PCP, μ_A^* , as well as the effect of adding N_A on both μ_A^* and μ_B^* . The last equality shows that whenever $\bar{V}_A = \bar{V}_B$, μ_A^r reduces to μ_A^* . The last form also contains the same term as advocated by Sharp et al. Therefore, it appears as if Tuñón et al. provide an *exact derivation* of Sharp et al.’s quantity. Equation 3.2 is indeed an exact deduction from (3.1). One can also form the difference for the two phases α and β , to obtain the quantity

$$\Delta \mu_A^r \equiv \mu_A^{r\beta} - \mu_A^{r\alpha} = \mu_A^{*\beta} - \mu_A^{*\alpha} + kT(r^\beta - r^\alpha) \quad (3.3)$$

Although the derivation of this quantity is exact, we claim that the quantity defined on the left-handside of (3.3) is not relevant to the solvation process and cannot be used as a SGE in those applications discussed in section 2. To see this, we first note the G^* defined in (3.1) is *not* the Gibbs energy of any physical system; hence, μ_A^r is not a chemical potential. Tuñón et al. do not explain the physical meaning of this quantity. They only interpret the quantity $\Delta \mu_A^r$ in (3.3) as being a “more general” one than $\Delta \mu_A^*$, in the sense that $\Delta \mu_A^r$ reduces to $\Delta \mu_A^*$ when $\bar{V}_A = \bar{V}_B$. This, in itself, does not confer any physical meaning to $\Delta \mu_A^r$.

To assign meaning to G^* and to μ_A^r , we note that G^* can be written as

$$G^* = G(T, V, N_A, N_B) - G^{\text{ig}}(T, V, N_A, N_B) \quad (3.4)$$

where G^{ig} is the Gibbs energy of the system (T, V, N_A, N_B), if it were an ideal gas, i.e., G^* is the change in the Gibbs energy for “turning on” *all* the interactions in a system at constant T, V, N_A, N_B . When taking the derivative with respect to N_A , one gets (3.2), a quantity which does include all *solute–solute*,

solute–solvent, and *solvent–solvent* interactions. Unfortunately, as we show below, this quantity, or the corresponding quantity $\Delta \mu_A^r$ is *not* relevant to the solvation process. It cannot be used either for calculating the solubility or the equilibrium constant ratio, as discussed in the previous section. Thus, although the derivation of $\Delta \mu_A^r$ by Tuñón et al. is indeed algebraically exact, it is a derivation of a quantity that has no relevance to solvation. (Tuñón et al. claimed that their quantity is useful for calculating the surface tension. We will show, however, in the next section that neither $\Delta \mu_A^r$ nor the correct solvation Gibbs energy $\Delta \mu_A^*$ can be used for that purpose).

The form of μ_A^r in eq 3.2 suggests that μ_A^r is somehow “more general” than μ_A^* since it includes μ_A^* as well as two additional quantities—the derivatives of μ_A^* and μ_B^* with respect to N_A . However, a more general quantity does not necessarily mean a meaningful SGE.

It is easy to construct many others, even more general quantities, that will include additional effects. One such quantity is the following: Suppose that we choose to view the same system as above, (assuming for definiteness that the solvent is water) as a mixture of solutes A, hydrogens H, and oxygens O. This is a perfectly legitimate point of view. (Another, even “more general” one will be a mixture of electrons and various nuclei). We can define a new quantity, similar to (3.1), by

$$G^{**} = N_A \mu_A^* + N_H \mu_H^* + N_O \mu_O^* \quad (3.5)$$

Its derivative with respect to N_A is

$$\mu_A^{rr} = \mu_A^* + N_A \frac{\partial \mu_A^*}{\partial N_A} + N_A \frac{\partial \mu_N^*}{\partial N_A} + N_O \frac{\partial \mu_O^*}{\partial N_A} \quad (3.6)$$

This is also an exact quantity, and certainly “more general” than G^* in (3.2), since it also includes the effect of adding N_A to μ_A^* , μ_H^* and μ_O^* . Therefore, although in some sense μ_A^{rr} is “superior” to μ_A^r and to μ_A^* , it is *not* the quantity required for a solvation Gibbs energy. The reason is the following: In the *solvation process*, we are required to “turn on” (or to couple) one solute A to the entire solvent. The solvent should be *fully coupled* before inserting the newly added solute A. In using μ_A^r , we start with a “solvent” which is uncoupled with respect to solvent–solvent interactions. Therefore, μ_A^r accounts not only for the coupling of A to the solvent but also for coupling all solvent–solvent interactions. Similarly, in using μ_A^{rr} , we start with a “solvent” with uncoupled hydrogens and oxygens; therefore, μ_A^{rr} accounts for coupling A to the solvent, coupling all solvent–solvent interactions as well as the interactions between hydrogens and oxygens. (Had we started with electrons and nuclei, we could have added also the interactions within all atoms in the solvent). All these are clearly generalization of μ_A^* , fortunately, they are *not required* in the solvation process of A in the (fully coupled) solvent.

One could have claimed that by using μ_A^r rather than μ_A^* one is simply choosing a different *reference system*. Thus, in our case, we define

$$\Delta \mu_A^* = \Delta G_A^* = \mu_A^{*l} - \mu_A^{*ig} \quad (3.7)$$

One can certainly choose any other *fixed* reference system, say pure hexane or pure water at the same T and P . Clearly, the choice of the ideal-gas phase is the simplest one. However, one *cannot* use the “ideal gas” phase of Tuñón et al. as a reference system. The reason is that the pressure of this “ideal gas” phase, defined by the variables T, V, N_A, N_B , is different from the pressure defined for the *real* system at T, V, N_A, N_B .

Thus we write, for clarity

$$G^* = G(T, V, N_A, N_B; P) - G^{\text{ig}}(T, V, N_A, N_B; P^*) \quad (3.8)$$

The pressure P^* is much larger than P . (Note that T , V , N_A and N_B are a sufficient number of independent variables to describe any two-component system. Here, however, the *real* and the *ideal* systems are two *different* systems and, therefore, in general will have difference pressures even though T , V , N_A , and N_B are fixed). Since the pressure P^* will depend on the system studied, the corresponding ideal gas system at $(T, V, N_A, \text{and } N_B; P^*)$ cannot serve as a *fixed* or a universal reference system. (Similarly, if we use G^{**} in (3.5), we shall obtain a new ideal-gas phase, at $(T, V, N_A, \text{and } N_B; P^{**})$, with P^{**} even a higher pressure than P^*).

We conclude that $\Delta\mu_{Ar}$ as derived by Tuñón et al. is not a SGE quantity, and it is not clear where such a quantity might be useful (see also next section).

We next turn to Kumar et al.'s recent paper.¹² This is more difficult to criticize since it contains several, not well-defined, quantities and a few false statements. The paper's abstract states that "The sweeping criticism of the applicability of the FH theory that appeared in recent literature are therefore not valid". This is a false and misleading statement! We have criticized the "correction" that Sharp et al. suggested to the SGE, based on FH theory. We have never criticized the "applicability of the FH theory". Thus, by changing the original issue, Kumar et al. mislead the reader to think that our original criticism is invalid. What they actually show in this paper is not that their original "corrected" equation is correct (which would invalidate our criticism), but that there exists *another equation* (see (3.11) below) that has a similar form, and for which our criticism does not apply and obviously is invalid. More specifically, the paper starts with the definition of a quantity referred to as "the free energy per unit area of a non polar surface in contact with water. Free energies are usually obtained from solute transfer experiments using the familiar expression"¹

$$\Delta G^0 = -RT \ln(\rho^{(2)}/\rho^{(1)}) \quad [1] \quad (3.9)$$

We use square brackets to denote the equation numbers as in Kumar et al.'s paper.¹²

We have never discussed the "free energy per unit area of a non polar surface in contact with water". Since Kumar et al. refer to ref 1 (in their paper), we presume that ΔG^0 is the same as our ΔG^* or $\Delta\mu^*$ that we have been using. The " Δ " in our ΔG^* stands, as usual, for the *difference* between the initial and final states of a system. It is not clear what are the initial and final states in their ΔG^0 , referred to above. We conjecture that what they meant is that ΔG^* (or ΔG^0) is entirely due to surface Gibbs energy. We shall discuss this assumption in the next section.

The main issue that was raised in ref 4 was that a relation of the form (3.9) (or (2.3)) were "correct only under special circumstances",⁴ and "when the solute and solvent molecules are of different size, an additional term enters into the analysis".⁴ In our criticism⁵ we have shown that, since (2.3) and (3.9) are exact and valid for any solute in any solvent, one does not need to "correct" this equation. The "corrected" quantity derived by Sharp et al. has the form

$$\Delta G^0 = -RT \ln(\rho^{(2)}/\rho^{(1)}) + RT(r^{(2)} - r^{(1)}) \quad (3.10)$$

Since we have proved⁵ that this "correction" is wrong, it follows that our original "sweeping criticism" is still valid!

In the more recent papers, Kumar et al. divert the attention of the reader to a new issue that did not appear in their original paper. They define a *new quantity* described by "the component of ΔG^0 that is dependent only on contact solute/solvent interaction", and they write the expression

$$\Delta G_{\text{int}}^0 = -RT \ln(\rho^{(2)}/\rho^{(1)}) + f(v_1 v_2 v_p) \quad [2a] \quad (3.11)$$

Regarding this and a similar equation [2b], the authors stated: "The issue that has raised... the disagreement is the proposed use of equation [2b] rather than equation [1] to define transfer free energies. Ben-Naim and Mazo¹⁰ have stressed that the separation of terms described by eq [1] is an exact result... Consequently these authors argue that the use of eq [2a] is incorrect".

This is a false and deceptive statement. Ben-Naim and Mazo⁶ claimed that (2.3) or (3.9) are exact and that the "correction" proposed by Sharp et al. is incorrect. They never claimed anything regarding the newly defined quantity ΔG_{int}^0 in [2a] and [2b].

Kumar et al. continue: "Once one understands the underlying issues it is of little consequence whether one uses eq. [1] or [2b]. In this light the emphatic negative response of Ben-Naim and Mazo,¹⁰ and Holtzer¹⁴ to the use of eq. [2b] thus seems somewhat extreme". This, again, is a false statement. Our response to [2b] was *not* emphatic, *not* negative, and *not* extreme. We have never responded to [2a] nor to [2b] *at all*. All our criticism was directed to (3.10), for which our response was indeed negative.

Finally, in their concluding section Kumar et al. state "Ben-Naim and Mazo insist on the use of equation [1], these authors nevertheless argue that volume dependent makes important contribution to transfer free energies. Thus although they do not point out, they apparently agree with the central conclusion of Sharp et al."

We have indeed insisted on using [1] (or (2.3)), in response to the original claim of Sharp et al. that (2.3) is incorrect for the case of when the sizes of the solute and solvent molecules are different. We have shown that, for any solute, of any volume, (2.3) is valid, and one need not add "corrections" due to difference in volumes. This is in sharp *disagreement* with the "central conclusion" of Sharp et al. Therefore, it is clear that we *do not* agree with their "central conclusion" as stated in the original paper (ref 4). It seems to us that Kumar et al., although asserting the contrary, are actually accepting *our* criticism and effectively admitting their error in their first paper.

4. Conclusion and Some Words of Warning

Nowadays, we feel confident that the quantity ΔG_A^* or $\Delta\mu_A^*$ as defined in section 1 is still the best quantity that measures the solvation Gibbs energy of a molecule A in any solvent. It is also the quantity required for calculating partition coefficients between two solvents or equilibrium constants in a liquid phase. According to Sharp et al.³ and, more recently, Tuñón et al.,¹¹ the newly defined quantity $\Delta\mu_A^r$ is "useful" for calculating the water-hydrocarbon surface tension. Therefore, they argue, this particular application justifies the usage of the quantity $\Delta\mu_{Ar}$.

Unfortunately, the later application of either $\Delta\mu_A^*$ or $\Delta\mu_A^r$ is not based on any well-established relationship between solvation Gibbs energy and surface tension. This is in contrast to the well established relationships between SGEs and partition coefficients, or equilibrium constants as discussed in section 2.

The application of SGEs to calculate surface tension was originally suggested by Tanford,¹⁵ who identified the hydrophobic effect with the work required to create a new surface between water and hydrocarbon. The proposed relationship is

$$\Delta\mu^* = \gamma A \quad (4.1)$$

where A is the surface area of the molecule and γ the surface tension. Using such a relationship, Tuñón et al. have succeeded in obtaining a remarkable agreement between the microscopic surface tension γ and the macroscopic surface tension.¹¹

One of us criticized this application in a recent article.¹⁴ The main points of this criticism are (1) No relationship of the form (4.1) between SGE and surface tension has ever been derived, only postulated. (2) Even if such a relationship could be shown to be a good approximation for $\Delta\mu^*$, the quantity γ , having the dimensions of surface tension, cannot be identified with the *macroscopic* surface tension without further demonstration. (3) The quantity, actually used in (4.1), was not $\Delta\mu^*$, but the quantity $\Delta\mu^r$, which we have shown to be irrelevant to solvation. (4) The surface area of a small molecule is not a well-defined quantity, and the quantity chosen by Tuñón et al. is therefore quite arbitrary.

Therefore, we feel that such an application of the SGE is not justified and certainly does not confer any meaning upon the quantity $\Delta\mu^r$. We therefore urge that the use of such a quantity to measure solvation Gibbs energy be abandoned.

The usage of the newly defined quantities, such as $\Delta\mu^r$, has caused further confusion in a related field, referred to vaguely as the hydrophobic ($H\phi O$) effect. When Kauzmann introduced the $H\phi O$ effect in 1959,¹⁶ he was cautious in making a tentative hypothesis that the $H\phi O$ might be important as a driving force for biochemical processes. Although no firm evidence was provided, this hypothesis was later accepted as being well established, and the $H\phi O$ effect is regarded in many articles and some text books as the most important driving force of such processes.

Recently^{2,17} a careful examination of various well-defined $H\phi O$ effects has shown that, first, Kauzmann's model of using transfer Gibbs energies is not appropriate for describing the transfer of $H\phi O$ groups into the interior of a protein. The more relevant quantity is the *conditional* SGE^{1,2} of the $H\phi O$ groups. Experimental data indicate that the conditional SGEs might be

far smaller than the bare (unconditional) SGEs. Second, several arguments were recently published indicating that various hydrophilic ($H\phi I$) effects might be large.^{2,18,19} Therefore, at present it seems that the $H\phi O$ effect hypothesis and its relative importance to biochemical processes is losing some ground in favor of the $H\phi I$ effects.

At this juncture there appears the paper by Sharp et al., which leads us to conclude that the $H\phi O$ effect has been *underestimated*, claiming that the $H\phi O$ effect is actually stronger than what we used to believe. They reached this conclusion by first identifying the $H\phi O$ effect with the SGE (which is already known to be inappropriate^{2,17}). Second, they used a conjectured relationship between the SGE and surface tension. Finally they used "corrected" values of the SGEs, not the correct values. These erroneously inflated values of the SGEs led them to conclude that the $H\phi O$ effect is actually larger or stronger than that previously estimated. This is certainly going to confuse the biochemists, especially at a time when there is growing evidence that the $H\phi O$ effects are probably *smaller* than those previously estimated and perhaps also smaller than the corresponding $H\phi I$ effects.

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