

## Thermal Equivalent Dilution

A. Braibanti,\* E. Fisicaro, and C. Compari

Pharmaceutical Department, Applied Physical Chemistry Section, University of Parma, I-43100 Parma, Italy

Received: April 14, 1998; In Final Form: July 10, 1998

By developing a statistical thermodynamic model for the equilibria in solution, in *nonreacting* systems, the correspondence between heat capacity and dilution entropy change produces *thermal equivalent dilution*, *TED*. The principle of thermal equivalent dilution is validated by statistical thermodynamic relationships. Examples are given of the interpretation of experimental data by means of TED.

In recent articles,<sup>1–6</sup> we have applied what we have called the principle of “*thermal equivalent dilution*” (*TED*), whereby one calculates the change of activity produced by a change of temperature

$$-\partial \ln a_A / \partial \ln T = C_{p,A} / R \quad (1)$$

It seems that this principle had never been applied before. To us it has offered the possibility of interpreting the change with temperature of several processes in aqueous solution, namely, the protonation constant of carboxylic acids,<sup>3</sup> the solubility of noble gases in water,<sup>4</sup> the solubility of organic liquids and gases in water,<sup>5</sup> and the denaturation of proteins.<sup>6</sup> We have now searched for a statistical thermodynamic foundation of this principle, in order to go beyond what seemed to be a personal assumption.

The relationship of eq 1 has been obtained by equating the change of entropy due to dilution or reciprocal activity (dilution  $d_A = \text{activity}^{-1}$ )

$$dS = -R d \ln a_A \quad (2)$$

to the change of entropy due to temperature

$$dS = C_{p,A} d \ln T \quad (3)$$

respectively.

The relationship between dilution and entropy expresses the property that a solute is going to distribute spontaneously over the maximum accessible volume. The accessible volume that can be measured by the dilution, i.e., by the reciprocal of concentration or activity, represents the state probability of the solution. If the concentration of A is expressed as mol dm<sup>-3</sup>,

$$[A] = n/V \quad (4)$$

then the volume accessible to a mole of substance is the dilution

$$d = V/n \quad (5)$$

Analogously, if an active concentration or activity  $a_A$  is substituted for  $[A]$ , the entropy probability change  $\Delta S = S_2 - S_1$  between two solutions of the same species is related to the ratio

$$d_2/d_1 = \exp(\Delta S/R) \quad (6)$$

which by differentiation of the relative probability yields eq 2.

On the microscopic molecular scale, the accessible volume of one molecule becomes

$$\phi_A = V/(N_L n) \quad (7)$$

where  $N_L$  is Avogadro's number. The probability of state of the same solutions of eq 6 is given on the microscopic scale by the ratio

$$\phi_2/\phi_1 = \exp(\Delta S/R) = \exp(\Delta s/k_B) \quad (8)$$

where  $k_B$  is the Boltzmann constant and  $\Delta s = \Delta S/N_L$ .

Equation 1 states that any increase of temperature is equivalent to an increase of the dilution of the solute, i.e., the volume accessible to the solute is increased. In terms of dynamic properties, the increase in temperature increases the speed of the solute and thus reduces the residence time of the particle in each cell of the volume. This is exactly the same result that can be achieved by diluting the solute by increasing the accessible volume.

If the system is *nonreacting*, i.e., it is characterized by a continuous distribution of enthalpies, the thermal coefficient, isobaric heat capacity can be expressed either as

$$C_{p,A} = \partial H / \partial T \quad (9)$$

or as

$$C_{p,A} = \partial S / \partial \ln T \quad (10)$$

In the former case it is related to the dispersion of population among enthalpy levels<sup>1,2</sup> and in the latter to the entropic multiplicity of states. The two situations are perfectly equivalent from a thermodynamic point of view and cannot be distinguished by thermodynamic means.

There is a correspondence between a change in energy and a change in entropy, as shown by the eqs 9 and 10. We can treat the heat capacity according to eq 10.

Both intensive variables, temperature  $T$  and dilution  $d_A = 1/[A]$ , produce entropic changes as shown by eqs 2 and 3. The total entropy change is

$$dS_A = -R d \ln[A] + C_{p,A} d \ln T \quad (11)$$

Equation 1, the second term of the right-hand side (rhs) of eq 11, can be considered as a virtual change of dilution  $d \ln[A_{\text{virt}}]$  or *thermal equivalent dilution* (*TED*)

$$dS_A = -R d \ln[A] - (\partial \ln a_A / \partial \ln T) d \ln T = -R d \ln[A] - R d \ln[A_{\text{virt}}] \quad (12)$$

The total entropy change can be considered as a change of activity

$$dS_A = -R d \ln\{[A][A_{\text{virt}}]\} = -R d \ln a_A \quad (13)$$

A demonstration on the molecular scale is the following. For  $n$  molecules of solute, one can write

$$-\Delta G/RT = \ln\{\phi_0 N_L / (\phi_m N_L n)\} = \ln(1/V_m)/(n/V_0) \quad (14)$$

where  $V_m = N_L \Lambda^3 = N_L (h^2/2\pi m k_B T)^{3/2}$  is the statistical average volume of 1 mol of ideal gas at any temperature, where  $\Lambda^3$  is De Broglie's volume,  $k_B$  is Boltzmann's constant,  $h$  is Planck's constant,  $m$  is the mass of the particle, and  $\phi_0$  and  $\phi_m$  are the microscopic accessible volumes of ideal gas and molecule A, respectively.

The free energy of mixing an ideal gas is directly transformed into an entropy of mixing

$$\Delta S/R = \ln\{(V_0/n)/(\phi_m N_L)\} = \ln\{(1/V_m)/[A]\} \quad (15)$$

where  $1/[A]$  has been substituted for  $(V_0/n)$ . By taking the logarithm of  $1/V_m$ , one obtains

$$\ln(N_L^{-1} \Lambda^{-3}) = \ln\{(h^2/2\pi m k_B N_L)^{-3/2}\} + (3/2) \ln T + \ln N_L^{-5/2} \quad (16)$$

and then

$$\Delta S/R = \ln\{(1/[A])\} + \ln\{(h^2/2\pi m R)^{-3/2}\} + (3/2) \ln T + \ln N_L^{-5/2} \quad (17)$$

which is the Sakur-Tetroole equation. By differentiation of eq 17 and by using  $C_{v,\text{id}} = (3/2)R$  for an ideal gas, one obtains

$$d(\Delta S) = R d \ln(1/[A]) + C_{v,\text{id}} d \ln T + R d \ln V_0 \quad (18)$$

By considering that from the equation of state of perfect gases at constant pressure  $d \ln V_0 = d \ln T$ , eq 18 yields

$$d(\Delta S) = R d \ln(1/[A]) + (3/2 + 1)R d \ln T \quad (19)$$

Then, by remembering that  $C_p = (5/2)R$  for the ideal gas represents the distribution of energy among the available degrees of freedom, one obtains by extension to real solutions

$$d(\Delta S) = R d \ln(1/[A]) + C_p d \ln T \quad (20)$$

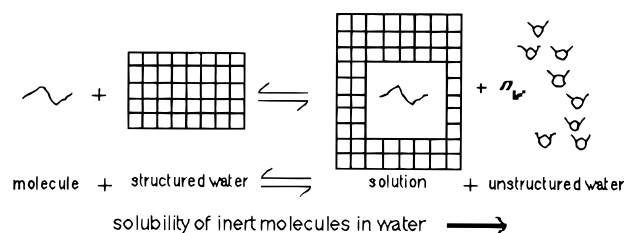
which is the sum of the two changes of entropy of eq 11. The experimental  $C_p$  represents the partition of energy among the real motion components of the solute particle. The second term of the rhs of eq 20, transformed into virtual dilution as the corresponding term of the eq 12, is equivalent to a temperature-dependent activity coefficient.

## Applications

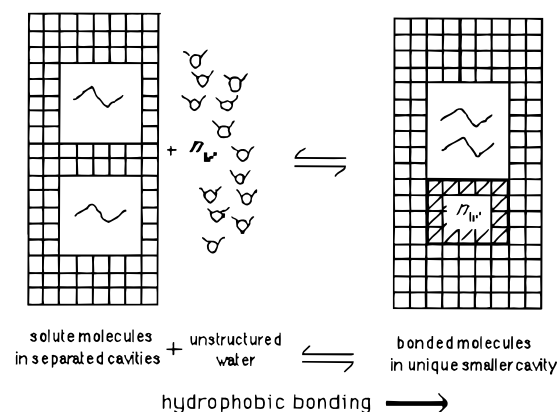
An important consequence of TED is its application to diluted systems, particularly aqueous solutions. In such systems, the solvent very often takes part in the reaction with  $n_w$  molecules giving rise to a free energy contribution<sup>3</sup>

$$d(-\Delta G_w/RT) = n_w d \ln[W] \quad (21)$$

The derivative of this free energy term with respect to the



**Figure 1.** Solubilization of inert molecules in water. The insertion of the solute into the cavity is exothermic but most of the heat released is absorbed by an amount  $n_w C_{p,w} T$  by the water molecules expelled, to achieve the kinetic energy necessary at the temperature  $T$ .



**Figure 2.** Hydrophobic bonding. The formation of an hydrophobic bond such as those that occur in folding of proteins is accompanied by the absorption of water molecules to fill that portion of the cavity which would be empty after the formation of the bonds.

reciprocal temperature yields an enthalpy contribution

$$\partial(-\Delta G_w/RT)/\partial(1/T) = -\Delta H_w/R \quad (22)$$

By substitution of eq 21 into eq 22 and by application of eq 1, this enthalpy term is dependent upon the temperature

$$-\Delta H_w = n_w C_{p,w} T \quad (23)$$

Therefore, the total apparent enthalpy that is determined at different temperatures, either obtained by calorimetry or by determination of the equilibrium constant plotted against  $T$ , is a straight line

$$\Delta H_{\text{app}} = \Delta H^\phi - \Delta H_w = \Delta H^\phi + n_w C_{p,w} T \quad (24)$$

where  $\Delta H^\phi$  is the "true" enthalpy change of the reaction.

At 298.15 K, the slope divided by  $C_{p,w} = 75.36 \text{ J K}^{-1} \text{ mol}^{-1}$  allows the determination of the number  $n_w$  of water molecules involved in the reaction.

Application of this principle to the protonation constants of carboxylic acids determined at different temperatures<sup>3</sup> has shown that for the protonation of several monocarboxylic acids the number of water molecules is practically constant,  $n_w = 2.1$ . Plots of the equilibrium constants of carboxylic acids plotted against  $(1/T)$  show, as is well-known, a minimum at temperatures near ambient. The fact that  $n_w$  is practically constant means that all the curves, appropriately displaced on the diagram, are superimposable and on the molecular scale that the mechanism is the same in every one of those acids.

The insertion of the solute into the cavity is exothermic, but most of the heat released is absorbed by an amount  $n_w C_{p,w} T$ ,

by the water molecules expelled, to achieve the kinetic energy necessary at the temperature  $T$ .

The TED principle has also been applied to the study of the solubility of noble gases in water at different temperatures,<sup>4</sup> where  $n_w = 1.55$  (He), 1.84 (Ne), 2.51 (Ar), 2.81 (Kr), 3.26 (Xe), 3.89 (Rn) is strictly proportional to the size of the dissolved monoatomic molecule. Moreover, the same principle can be applied to the interpretation of the solubility in water at different temperatures of liquid and inert gases<sup>5</sup> with a number of water molecules released ranging from  $n_w = 2.8$  ( $\text{CH}_4$ ) to  $n_w = 7.7$  ( $((\text{CH}_2=\text{CH}-)_2)$ ). All these examples can be related to the formation of a cavity in the structure of the solvent from which a number  $n_w$  of water molecules is expelled, where  $n_w$  is proportional to the size of the inserted molecule (Figure 1).

The same TED principle can explain the denaturation enthalpy of proteins, which has been found to be linearly dependent upon the temperature. The values  $n_w$  obtained for proteins are much higher than for noble gases because the size of the molecules is increased.<sup>6</sup> For some lysozymes, the number of water molecules released in the denaturation ranges from  $n_w = 89$  for hen egg white lysozyme with molar mass 14 100 Da to  $n_w$

$= 122$  for wild T4 lysozyme with molar mass 18 700 Da. Higher values of  $n_w$  are obtained when mutants of wild T4 are studied where hydrophobic groups are introduced.

The relevance of the general mechanism of cavity formation for a new interpretation of the "hydrophobic bond" is clearly apparent. In fact, the renaturation of protein ( $n_w < 0$ ) implies the restructuring of water molecules to fill the cavity, which is actually smaller after the formation of a hydrophobic bond (Figure 2).

## References and Notes

- (1) Braibanti, A.; Fisicaro, E.; Dallavalle, F.; Lamb, J. D.; Oscarson, J. L. *J. Phys. Chem.* **1993**, *97*, 8054–8061.
- (2) Braibanti, A.; Fisicaro, E.; Dallavalle, F.; Lamb, J. D.; Oscarson, J. L. *J. Phys. Chem.* **1993**, *97*, 8062–8070.
- (3) Braibanti, A.; Fisicaro, E.; Dallavalle, F.; Lamb, J. D.; Oscarson, J. L.; Ughi, F. X. *J. Phys. Chem.* **1993**, *97*, 8071–8081.
- (4) Braibanti, A.; Fisicaro, E.; Dallavalle, F.; Lamb, J. D.; Oscarson, J. L.; Sambasiva Rao, R. *J. Phys. Chem.* **1994**, *98*, 626–634.
- (5) Braibanti, A.; Fisicaro, E.; Ghiozzi, A.; Compari, C.; Panelli, M. *J. Solution Chem.* **1995**, *24*, 703–717.
- (6) Braibanti, A.; Fisicaro, E. *Thermochim. Acta* **1994**, *241*, 131–156.