Calculation of Standard Formation Properties of Species from Standard Transformed Formation Properties of Reactants in Biochemical Reactions at Specified pH

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Up until now, standard transformed formation properties of biochemical reactants at a specified pH have largely been calculated from $\Delta_f G°$ and $\Delta_f H°$ values of the species that make them up. The equations for doing this have been derived by using a Legendre transform to define a transformed Gibbs energy G' that provides the criterion for equilibrium and spontaneous change at specified temperature T, pressure P, and pH. In the future, the $\Delta_f G°$ and $\Delta_f H°$ values of organic species in aqueous solutions will increasingly be calculated from the standard transformed properties $\Delta_f G'°$ and $\Delta_f H'°$ obtained from the apparent equilibrium constants of biochemical reactions and their temperature coefficients or apparent heats of reaction measured at a specified pH. It is therefore important to consider the inverse Legendre tranform that defines the Gibbs energy G in terms of the transformed Gibbs energy G' and the equations for calculating the $\Delta_f G°$ and $\Delta_f H°$ values of species from thermodynamic measurements at specified T, P, and pH. The use of these equations is illustrated.

Introduction

The thermodynamics of biochemical reactions can be discussed in terms of species, but, for many purposes, it is better to discuss these reactions at a specified pH in terms of reactants, that is, in terms of sums of species differing with respect to the numbers of hydrogen atoms they contain. This latter point of view also corresponds more closely to the study of enzyme-catalyzed reactions in the laboratory. When a reaction is discussed in terms of species at specified temperature T and pressure P, the Gibbs energy G provides the criterion for equilibrium, and measurements of the equilibrium constant K over a range of temperatures provide the means for determining the various standard formation properties of the species. However, when a biochemical reaction is discussed in terms of reactants, such as adenosine triphosphate (ATP), at a specified pH, the transformed Gibbs energy G' provides the criterion for equilibrium and spontaneous change. In this case, measurements of the apparent equilibrium constant K' over a range of temperatures at a specified pH provide the means for determining the standard transformed formation properties of the reactants, as well as the change in binding of hydrogen ions in the biochemical reaction at that pH. These two thermodynamic representations of a system are related by a Legendre transform that ensures that no information is lost in replacing an extensive variable by an intensive variable[1]. The transformed Gibbs energy G' is defined by the Legendre transform[2−6]

$$G' = G - n_c(\mathrm{H})\,\mu(\mathrm{H}^+) \tag{1}$$

where the conjugate variables are the amount $n_c(\mathrm{H})$ of the hydrogen component in the system and the specified chemical potential $\mu(\mathrm{H}^+)$ of hydrogen ions. The amount of the hydrogen component is the total amount of hydrogen atoms in the system. The point of view taken in writing eq 1 is that thermodynamic properties of species can be used to calculate thermodynamic properties of reactants at a specified pH. This approach has led to the calculation of standard transformed Gibbs energies of formation $\Delta_f G'°$ and standard transformed enthalpies of formation $\Delta_f H'°$ at pH 7 of about 100 biochemical reactants[7,8] for which $\Delta_f G°$ and $\Delta_f H°$ values are available for all the species that have significant concentrations at this pH. This just about exhausts the resources of $\Delta_f G°$ and $\Delta_f H°$ values for species of biochemical interest, but many more values of $\Delta_f G°$ and $\Delta_f H°$ for species in biochemical reactions can be calculated from the apparent equilibrium constants of some 500 enzyme-catalyzed reactions that have been critically evaluated by Goldberg and Tewari.[9−13]

When the primary source of thermodynamic data is values of the $\Delta_f G'°$ and $\Delta_f H'°$ of reactants, the new point of view corresponds with the use of the inverse Legendre transform

$$G = G' + n_c(\mathrm{H})\,\mu(\mathrm{H}^+) \tag{2}$$

As emphasized by Callen,[1] no thermodynamic information is lost in making either of these transformations. Therefore, it is important to examine the effect of this inverse Legendre transform on the fundamental equation for the transformed Gibbs energy G'.

Effect of the Inverse Legendre Transform

Consider a reaction system that contains N species and N' reactants, where a reactant is the sum of species that differ only in the number of hydrogen atoms they contain. The species are numbered $i = 1, 2, 3, ..., N$, and the reactants are numbered $j = 1, 2, 3 ..., N'$. When the reaction system is considered at a specified temperature T, pressure P, and pH, the criterion for equilibrium and spontaneous change is provided by the transformed Gibbs energy G', for which the fundamental equation is[2−4]

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$$dG' = -S' dT + V dP + \sum_{j=1}^{N'} \mu_j' dn_j' + RT \ln(10) n_c(H) dpH \quad (3)$$

This equation is important because it provides a means for deriving the expression for the apparent equilibrium constant K' for a biochemical reaction in terms of reactants, a number of Maxwell relations, and a Gibbs−Duhem equation. S' is the transformed entropy of the system that is defined by

$$S' = S - n_c(H) \bar{S}(H^+) \quad (4)$$

where S is the entropy of the system, $n_c(H)$ is the amount of the hydrogen component in the system, and $\bar{S}(H^+)$ is the molar entropy of hydrogen ions at the pH of the system. The amount of the hydrogen component is given by

$$n_c(H) = \sum_{i=1}^{N} N_H(i)\, n_i \quad (5)$$

where $N_H(i)$ is the number of hydrogen atoms in species i and n_i is the amount of species i. In eq 3, μ_j' is the transformed chemical potential of reactant j and n_j' is the amount of reactant j (a sum of species). The transformed chemical potential μ_i' of a species is given by

$$\mu_i' = \mu_i - N_H(i)\, \mu(H^+) \quad (6)$$

where $\mu(H^+)$ is the chemical potential of hydrogen ions at the specified pH. The transformed chemical potential μ_i' of species that differ only in the number of hydrogen atoms they contain are equal at chemical equilibrium, and so, the transformed chemical potential μ_j' of a reactant is equal to the transformed chemical potentials of the species it contains.

Before the inverse Legendre transformation of eq 3 is allowable, it is necessary to eliminate dpH in eq 3 in favor of $d\mu(H^+)$ and dT. The chemical potential of hydrogen ions depends on both the temperature and the concentration of hydrogen ions, and so

$$d\mu(H^+) = \left[\frac{\partial \mu(H^+)}{\partial T}\right]_{[H^+]} dT + \left[\frac{\partial \mu(H^+)}{\partial [H^+]}\right]_T d[H^+] = -\bar{S}(H^+)\, dT - RT \ln(10)\, dpH \quad (7)$$

Using this equation to eliminate dpH from eq 3 yields

$$dG' = -S' dT + V dP + \sum_{j=1}^{N'} \mu_j\, dn_j' - n_c(H)\, \bar{S}(H^+)\, dT - n_c(H)\, d\mu(H^+) \quad (8)$$

Substituting eq 4 yields

$$dG' = -S\, dT + V\, dP + \sum_{j=1}^{N'} \mu_j' dn_j' - n_c(H)\, d\mu(H^+) \quad (9)$$

Now the inverse Legendre transform given in eq 2 is used to introduce $n_c(H)$ as a natural variable of G, in exchange for $\mu(H^+)$. Taking the differential of G in eq 2 and eliminating dG' by the use of eq 9 yields

$$dG = -S\, dT + V\, dP + \sum_{j=1}^{N'} \mu_j' dn_j' + \mu(H^+)\, dn_c(H) \quad (10)$$

Introducing eqs 5 and 6 yields

$$dG = -S\, dT + V\, dP + \sum_{i=1}^{N} \mu_i\, dn_i \quad (11)$$

This is the fundamental equation for G for the system described in terms of species at specified T and P. This equation was originally the source of eq 3, and so, this derivation confirms that no thermodynamic information was lost in making the two successive Legendre transforms.

Calculation of $\Delta_f G°$ Values for Species of a Reactant from $\Delta_f G'°$ of the Reactant

At an earlier time, methods were developed for calculating the $\Delta_f G°$ and $\Delta_f H°$ values for species from measurements of K' and $\Delta_r H'°$ at a specified pH. The thermodynamic treatment of the ATP series[3] provides an example of this. The new issue that is discussed here is the calculation of the $\Delta_f G°$ and $\Delta_f H°$ values of species directly from the $\Delta_f G'°$ and $\Delta_f H'°$ values of the reactant they make up at the specified pH. In earlier calculations of the $\Delta_f G°$ and $\Delta_f H°$ from data on enzyme-catalyzed reactions, measurements of K' were used to calculate the equilibrium constant K for a reference chemical reaction, and this was used to calculate the $\Delta_f G°$ and $\Delta_f H°$ for a single species in the reference reaction for which these values were not known. This section is oriented toward calculations based on the $\Delta_f G'°$ and $\Delta_f H'°$ for reactants rather than on the $\Delta_r G'°$ and $\Delta_r H'°$ for a biochemical reaction.

The standard transformed Gibbs energies of formation $\Delta_f G'°$ of reactants can be calculated from measurements of apparent equilibrium constants K' by using

$$\Delta_r G'° = -RT \ln K' = \sum \nu_j' \Delta_f G_j'° \quad (12)$$

where ν_j' is the stoichiometric number of reactant j in an enzyme-catalyzed reaction. The prime is used to distinguish ν_j' from the ν_i of species in the underlying chemical reactions.

If a reactant consists of a single species at the specified pH, the $\Delta_f G_j'°$ of the reactant is given by eq 6, which can be written in the form

$$\Delta_f G_i'° = \Delta_f G_i° - N_H(i)\{\Delta_f G°(H^+) + RT \ln [H^+]\} \quad (13)$$

where the $\Delta_f G_i°$ is the standard Gibbs energy of formation of species i and $N_H(i)$ is the number of hydrogen atoms in species i. If a reactant consists of a single species, $\Delta_f G_j'° = \Delta_f G_i'°$. It is convenient to take these standard Gibbs energies of formation to be functions of the ionic strength rather than to deal with activity coefficients.[16,17] The standard Gibbs energy of the hydrogen ion $\Delta_f G°(H^+)$ is equal to zero at zero ionic strength but not at higher ionic strengths. When the $\Delta_f G_j'°$ for a reactant consisting of a single species can be calculated from a measured apparent equilibrium constant by use of eq 12, the standard Gibbs energy of the species $\Delta_f G_i°$ making up the reactant can be calculated using

$$\Delta_f G_i° = \Delta_f G_j'° + N_H(i)\{\Delta_f G°(H^+) + RT \ln [H^+]\} \quad (14)$$

When a biochemical reactant consists of two or more species, the species form a pseudoisomer group at a specified pH, and the standard transformed Gibbs energy of formation of reactant j at that pH is given by[2,14]

$$\Delta_f G_j'° = -RT \ln \sum \exp(-\Delta_f G_i'°/RT) \quad (15)$$

This is a kind of partition function. The term for the species with the fewest hydrogen atoms (species 1) can be taken out of the summation, and eq 15 can be rewritten as[15]

$$\Delta_f G_j'^\circ = \Delta_f G_1'^\circ - RT \ln\left(1 + \frac{[H^+]}{K_1} + \frac{[H^+]^2}{K_1 K_2} + ...\right) \quad (16)$$

where K_1, K_2, ... are the successive acid dissociation constants. If the $\Delta_f G_j'^\circ$ can be determined experimentally as a function of pH, this calculation can be made by the use of linear regression. As a simple example, consider that the biochemical reactant at the specified pH is made up of A^- and HA so that

$$\Delta_f G'^\circ(A) = \Delta_f G'^\circ(A^-) - RT \ln\left(1 + \frac{[H^+]}{K(HA)}\right) = \Delta_f G^\circ(A^-) - N_H(A^-)\{\Delta_f G^\circ(H^+) + RT \ln [H^+]\} - RT \ln\left(1 + \frac{[H^+]}{K(HA)}\right) \quad (17)$$

where A in $\Delta_f G'^\circ(A)$ represents the sum of A^- and HA. When the $\Delta_f G'^\circ(A)$ has been obtained from a measured value of K', the value of the $\Delta_f G^\circ(A^-)$ can be calculated, provided the acid dissociation constant of HA is known, by use of

$$\Delta_f G^\circ(A^-) = \Delta_f G'^\circ(A) + N_H(A^-)\{\Delta_f G^\circ(H^+) + RT \ln [H^+]\} + RT \ln\left(1 + \frac{[H^+]}{K(HA)}\right) \quad (18)$$

To calculate the standard Gibbs energy of formation of the weak acid, the expression for the $\Delta_r G^\circ(HA = H^+ + A^-)$ can be solved to obtain

$$\Delta_f G^\circ(HA) = \Delta_f G^\circ(H^+) + \Delta_f G^\circ(A^-) + RT \ln K(HA) \quad (19)$$

After the $\Delta_f G^\circ(A^-)$ has been calculated using eq 18, this equation makes it possible to calculate the $\Delta_f G^\circ(HA)$.

It is convenient to take the standard thermodynamic properties in biochemical thermodynamics to be functions of the ionic strength so that complicated calculations with activity coefficients can be avoided in using tables. According to the extended Debye−Hückel theory[16,17] at 298 K, the standard Gibbs energy of formation of a species at ionic strength I can be calculated using

$$\Delta_f G_i^\circ(I) = \Delta_f G_i^\circ(I = 0) - 2.91482 z_i^2 I^{1/2}/(1 + BI^{1/2}) \quad (20)$$

where z_i is the charge number of the species and $B = 1.6\ L^{1/2}\ mol^{-1/2}$. This involves an approximation that can only be removed by using an equation with more parameters that depend on the ion species in the buffer.[18]

Calculation of the $\Delta_f H^\circ$ Values for Species of a Reactant from the $\Delta_f H'^\circ$ of the Reactant

The standard transformed enthalpy of formation $\Delta_f H_j'^\circ$ of a reactant at a specified pH can be calculated from measurements of temperature coefficients of K' or calorimetric measurements using

$$\Delta_r H'^\circ = \sum \nu_j' \Delta_f H_j'^\circ \quad (21)$$

where ν_j' is the stoichiometric coefficient of reactant j in the biochemical reaction. If a reactant consists of a single species at the specified pH, the standard enthalpy of formation of the species can be calculated by using

$$\Delta_f H_i^\circ = \Delta_f H_i'^\circ + N_H(i)\, \Delta_f H^\circ(H^+) \quad (22)$$

where $\Delta_f H_i'^\circ$ is the standard transformed enthalpy of formation of reactant j obtained by use of eq 21. The standard enthalpy of formation of the hydrogen ion is equal to zero at zero ionic strength but not at higher ionic strengths.

When a reactant consists of two or more species at the specified pH, the standard transformed enthalpy of formation of the reactant at that pH is given by

$$\Delta_f H_j'^\circ = \sum r_i \Delta_f H_i'^\circ \quad (23)$$

where the equilibrium mole fractions r_i of species in the reactant are given by[2,14]

$$r_i = \exp[(\Delta_f G_j'^\circ - \Delta_f G_i'^\circ)/RT] \quad (24)$$

The relation between the $\Delta_f H_j'^\circ$ of a reactant at a specified pH and the $\Delta_f H_i^\circ$ values of the species involved can be derived by using the Gibbs−Helmholtz equation for the transformed formation properties:[16]

$$\Delta_f H_j'^\circ = -T^2\left(\frac{\partial[\Delta_f G_j'^\circ/T]}{\partial T}\right)_{P,pH} \quad (25)$$

The use of this equation can be illustrated by considering the simple example of a biochemical reactant made up of A^- and HA. Substituting eq 17 for reactant A in eq 25 yields

$$\Delta_f H'^\circ(A) = \Delta_f H'^\circ(A^-) + RT^2\left(\frac{\partial \ln\{1+[H^+]/K(HA)\}}{\partial T}\right)_{P,pH} \quad (26)$$

Carrying out the differentiation yields

$$\Delta_f H'^\circ(A) = \Delta_f H'^\circ(A^-) - RT^2\left(\frac{[H^+]/K(HA)}{1 + [H^+]/K(HA)}\right)\left(\frac{\partial \ln K(HA)}{\partial T}\right)_P \quad (27)$$

Because

$$RT^2\left(\frac{\partial \ln K(HA)}{\partial T}\right)_P = \Delta_r H_{dis}^\circ \quad (28)$$

where $\Delta_r H_{dis}^\circ$ is the change in enthalpy in the acid dissociation, eq 27 can be simplified to

$$\Delta_f H'^\circ(A^-) = \Delta_f H'^\circ(A) + \left(\frac{[H^+]/K(HA)}{1 + [H^+]/K(HA)}\right)\Delta_r H_{dis}^\circ \quad (29)$$

Equation 22 shows that

$$\Delta_f H^\circ(A^-) = \Delta_f H'^\circ(A) + \left(\frac{[H^+]/K(HA)}{1 + [H^+]/K(HA)}\right)\Delta_r H_{dis}^\circ + N_H(A^-)\Delta_f H^\circ(H^+) \quad (30)$$

which shows that $\Delta_f H^\circ(A^-)$ can be calculated from $\Delta_f H'^\circ(A)$ at a specified pH only when $K(HA)$ and $\Delta_r H_{dis}^\circ$ are known for the weak acid.

The value of $\Delta_f H^\circ(HA)$ can be calculated from the expression for $\Delta_r H_{dis}^\circ$:

$$\Delta_f H^\circ(HA) = \Delta_f H^\circ(A^-) + \Delta_f H^\circ(H^+) - \Delta_r H_{dis}^\circ \quad (31)$$

Equation 29 shows that $\Delta_f H'^\circ(A^-)$ can be calculated when $\Delta_f H'^\circ(A)$, K_a, and $\Delta_r H_{dis}^\circ$ are known. $\Delta_f H^\circ(HA)$ can then be calculated using eq 31. The value of $\Delta_f H^\circ$ for a species can be adjusted to the desired ionic strength by use of[16,17]

$$\Delta_f H_i^\circ(I) = \Delta_f H_i^\circ(I = 0) + 1.4775 z_i^2 I^{1/2}/(1 + BI^{1/2}) \quad (32)$$

Calculation of Standard Gibbs Energies of Formation of Species of D-Sorbitol and D-Sorbitol 6-Phosphate at Three Ionic Strengths

For D-sorbitol, experimental measurements of the apparent equilibrium constant of the D-sorbitol dehydrogenase reaction (EC 1.1.99.21)[19]

$$\text{D-sorbitol} + NAD_{ox} = \text{D-fructose} + NAD_{red} \quad (33)$$

at 298 K and pH 7 have yielded[8] the values of $\Delta_f G'^\circ$ given in Table 1A. For D-sorbitol 6-phosphate, experimental measurements of the apparent equilibrium constant of the D-sorbitol 6-phosphate 2-dehydrogenase reaction (EC 1.1.1.140)

$$\text{D-sorbitol 6-phosphate} + NAD_{ox} = \text{D-fructose 6-phosphate} + NAD_{red} \quad (34)$$

at 298 K and pH 7 have yielded[8] the values of $\Delta_f G'^\circ$ given in Table 1A. The calorimetric enthalpy of reaction for the D-sorbitol dehydrogenase reaction has been determined by Tewari and Goldberg[20] at 298.15 K; pH 7, and 0.217 M ionic strength to be 17.1 ± 0.3 kJ mol^{-1}. From this study, they have calculated the standard enthalpy of formation of D-sorbitol to be -1312.62 kJ mol^{-1} and the standard transformed enthalpy of formation $\Delta_f H'^\circ$ at 298 K, pH 7, and $I = 0.25$ M to be -1318.36 kJ mol^{-1}. It will not be possible to calculate the $\Delta_f H^\circ$ for the species of D-sorbitol 6-phosphate until reaction 34 or some other reaction involving this reactant has been studied calorimetrically.

Because D-sorbitol is a single species, eq 14 yields the values of the $\Delta_f G^\circ$ at the three ionic strengths that are given in Table 1B. This species is uncharged and contains 14 hydrogen atoms. At pH 7, D-sorbitol 6-phosphate is made up of two species, $S6P^{2-}$ and $HS6P^-$, and so, eqs 18 and 19 are used to calculate the $\Delta_f G^\circ$ values for these two species. Because K(HA) has not been determined for $HS6P^-$, it is assumed that it is the same as that for glucose 6-phosphate, that is, 3.795×10^{-7} at 298 K and zero ionic strength.[3] The species $S6P^{2-}$ contains 13 hydrogen atoms, and so at zero ionic strength, eq 18 yields the value of the $\Delta_f G^\circ(S6P^{2-})$ that is given in Table 1B. Equation 19 yields the value of the $\Delta_f G^\circ(HS6P^-)$ at zero ionic strength. Values at higher ionic strengths are calculated using eq 20.

Table 1 makes it possible to calculate equilibrium constants for the following reactions at 298.15 K and ionic strengths of 0, 0.10, and 0.25 M:

$$\text{sorbitol 6-phosphate} + H_2O = \text{sorbitol} + P_i \qquad K'(\text{pH } 7) = \{1527, 1043, 947\} \quad (35)$$

$$S6P^{2-} + H_2O = \text{sorbitol} + HPO_4^{2-} \qquad K = \{734, 734, 734\} \quad (36)$$

$$HS6P^- + H_2O = \text{sorbitol} + H_2PO_4^- \qquad K = \{4605, 4605, 4605\} \quad (37)$$

Discussion

Legendre transforms are important in thermodynamics because they provide a means for introducing intensive variables as natural variables. If a thermodynamic potential can be expressed as a function of its natural variables, all of the thermodynamic properties of the system can be calculated. The use of a Legendre transform ensures that no information is lost. Inverse Legendre transforms can be used to replace intensive natural variables with extensive natural variables. In this paper, the pH in the fundamental equation for G' is replaced by the amount of the hydrogen component. This is of interest in biochemical thermodynamics when transformed formation properties at a specified pH are available, but the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ of species involved in a reactant are needed. When a reactant is a single species, the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ can be calculated from the transformed properties at a specified pH using eqs 14 and 22. When a reactant is made up of two species, the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ of the more basic form can be calculated using eqs 18 and 30. The values for the more acidic form can be calculated using eqs 19 and 31. When the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ can both be calculated for a species, the $\Delta_f S^\circ$ can also be calculated.

TABLE 1. Experimental Values of $\Delta_f G'^\circ$ at 298 K and pH 7 for Reactants and Calculated Values of $\Delta_f G^\circ$ for Species at 298 K at Three Ionic Strengths

	ionic strength (kJ/mol)		
	0 M	0.10 M	0.25 M
	A. Reactants		
D-sorbitol	−388.92	−380.35	−377.58
D-sorbitol 6-phosphate	−1272.01	−1266.24	−1264.42
	B. Species		
D-sorbitol	−948.31	−948.31	−948.31
$S6P^{2-}$	−1790.86	−1793.31	−1794.10
$HS6P^-$	−1827.51	−1828.12	−1828.32

The use of apparent equilibrium constants of enzyme-catalyzed reactions is going to be of increasing importance for the determination of the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ of species in aqueous solutions because of the problem of nonspecificity that is characteristic of classical methods. When complicated organic molecules undergo reactions in aqueous solutions, there may be competing reactions that yield complicated mixtures at equilibrium. This makes it difficult to obtain accurate equilibrium constants in terms of species. When organic reactions are studied calorimetrically, the production of complicated mixtures also makes it very difficult to obtain accurate values of the $\Delta_f H^\circ$ for the species produced. The advantage of using enzyme-catalyzed reactions is that very specific products are obtained. This article deals with the calculation of the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ of the species involved in biochemical reactions. These thermodynamic properties are of special interest in understanding the mechanisms of enzyme-catalyzed reactions because the mechanism needs to be described in terms of species rather than reactants. In addition to measurements of apparent equilibrium constants at a specified pH, calculations of the $\Delta_f G^\circ$ and $\Delta_f H^\circ$ of species require information on the acid dissociation constants of the reactants.

The opportunities to obtain standard thermodynamic properties of reactants in biochemical reactions are great but so is the need for more measurements of apparent equilibrium constants and heats of reaction. About 3500 enzymes have been assigned names and numbers,[19] but apparent equilibrium constants have been measured for only about 500 of these reactions.[9−13] The 500 reactions that have been studied can yield the $\Delta_f G'^\circ$ and $\Delta_f H^\circ$ for about 1000 reactants, but the number of reactants for which these standard thermodynamic properties are needed is of the order of 10 000. The number of reactants here is approximate because some enzymes catalyze more than one reaction.

Calculations of the $\Delta_f G°$ and $\Delta_f H°$ have been described here in a practical way, but it is important to understand that there are some problems with accuracy in addition to problems in the accuracy of the measurements of the apparent equilibrium constants and the purities of the reactants. One problem is caused by the formation of complex ions between species of the reactants and metal ions in the buffer. More data are needed on equilibrium constants for the formation of complex ions. At the present time, there is so little data on these constants and on actual experimental conditions in the enzymatic experiments that the binding of ions such as Mg^{2+} and Ca^{2+} are ignored, except in the case of the ATP series where there have been more studies.[3] The effects of the binding of metal ions may nearly cancel each other out if they are bound to the same extent by reactants and products. The basic problem is the difficulties of interionic attraction theory in the physiological range of ionic strengths, about 0.10−0.25 M. The current calculations are based on the use of the extended Debye−Hückel theory with $B = 1.6\ L^{1/2}\ mol^{-1/2}$ at 298 K. More accurate calculations will require the use of more complicated empirical equations that involve concentrations of specific ions in the buffer.[18]

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