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Recommendations for terminology and databases for biochemical thermodynamics ☆

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ABSTRACT

Chemical equations are normally written in terms of specific ionic and elemental species and balance atoms of elements and electric charge. However, in a biochemical context it is usually better to write them with ionic reactants expressed as totals of species in equilibrium with each other. This implies that atoms of elements assumed to be at fixed concentrations, such as hydrogen at a specified pH, should not be balanced in a biochemical equation used for thermodynamic analysis. However, both kinds of equations are needed in biochemistry. The apparent equilibrium constant K' for a biochemical reaction is written in terms of such sums of species and can be used to calculate standard transformed Gibbs energies of reaction $\Delta_r G'^\circ$. This property for a biochemical reaction can be calculated from the standard transformed Gibbs energies of formation $\Delta_f G_i'^\circ$ of reactants, which can be calculated from the standard Gibbs energies of formation of species $\Delta_f G_j^\circ$ and measured apparent equilibrium constants of enzyme-catalyzed reactions. Tables of $\Delta_r G'^\circ$ of reactions and $\Delta_f G_i'^\circ$ of reactants as functions of pH and temperature are available on the web, as are functions for calculating these properties. Biochemical thermodynamics is also important in enzyme kinetics because apparent equilibrium constant K' can be calculated from experimentally determined kinetic parameters when initial velocities have been determined for both forward and reverse reactions. Specific recommendations are made for reporting experimental results in the literature.

1. Preamble

Although thermodynamic considerations affect many aspects of biochemistry, including kinetic analysis of enzyme-catalyzed reactions, design and use of buffers for controlling the pH or pMg, identification of reversible steps in metabolic pathways, microcalorimetry, etc., there are some respects in which biochemical practice requires standardization with thermodynamic principles, the design of experiments, nomenclature, and the manner in which results are reported. Neglect of these factors can create problems for the analysis and comparison of the results of biochemical data. For example, inappropriate buffers or inappropriate experimental designs may have been used for controlling the concentrations of biochemically important ionic reactants, such as ATP, and especially the ion $MgATP^{2-}$. Additionally, different conventions may have been used in regards to standard states, concentration of water as a reactant, etc. In an early attempt to remedy this situation, the Inter-Union Commission on Biothermodynamics set up jointly by IUPAC, IUB, and IUPAB published *Recommendations for Measurements and Presentation of Biochemical Equilibrium Data* [1] in 1976, slightly revised in 1985 as *Recommendations for the Presentation of Thermodynamic and Related Data in Biology* [2]. Subsequently, IUPAC in conjunction with IUBMB published *Recommendations for Nomenclature and Tables in Biochemical Thermodynamics* [3] in 1994, in which the major innovation was the introduction of transformed thermodynamic properties at specified pH and pMg. Further development of transformed thermodynamic properties since then has led to new applications and this document, prepared under the auspices of the IUBMB, has two principal aims:

(1) to provide a concise update to the scientific formalism that underlies the application of transformed thermodynamic properties to biochemical thermodynamics, and

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(2) to update some of the recommendations given in 1994, with particular attention to the reporting of experimental results.

2. Introduction

Thermodynamics as normally applied to biochemical systems differs from chemical thermodynamics in allowing the pH to be specified in addition to the temperature and pressure. The importance of this difference can be illustrated by the consideration that the standard state of a solute is defined in chemical thermodynamics as a hypothetical ideal solution at a molality of 1 mol kg^{-1} [4]. If this were done for the hydrogen ion in biochemical reactions, it would make it impossible to form any impression of behavior at neutral pH just from knowledge of the standard thermodynamic properties of the species involved; one would also need knowledge of the acid dissociation constants of all the species that ionized between pH 0 and pH 7. In practice, therefore, biochemists have long considered the standard state of the hydrogen ion to be $1{\cdot}10^{-7}$ mol L^{-1}, and the standard state of H_2O is often treated as the concentration that actually exists in the system. The practical effect of the first point is that an equilibrium constant K' defined at any pH is related in a simple way to the corresponding Gibbs energy of reaction according to Eq. (2-7) below. In some biochemical publications the prime is omitted for the equilibrium constant, written simply as K, but included for the standard transformed Gibbs energy $\Delta_rG'°$. However, for the sake of consistency and greater clarity, the prime will be included for equilibrium constants in this document, and the standard transformed Gibbs energy $\Delta_rG'°$ will be written with a subscript r. In addition, the qualification "transformed" will be used to underline the fact that an equilibrium constant K' is a transformed form of the equilibrium constant K. In most biochemical contexts the transformation is assumed, and does not need to be explicit, but it is useful to include it in any comparison of biochemical and chemical conventions.

For reactions occurring in aqueous media, the practical effect of defining the standard state of H_2O as the pure solvent is that H_2O can be omitted from expressions for equilibrium constants. However, common practice is inconsistent in this respect: an expression for the chemical reaction catalyzed by an enzyme will sometimes include H_2O as a reactant, but omit it from the expression for the Gibbs energy of reaction, and sometimes also from the expression for the equilibrium constant.

Chemical thermodynamics treats ions that differ in their degree of protonation as separate species, but it becomes very complicated if one tries to do this with all reactants in an enzyme-catalyzed reaction that have significant concentrations in the pH range considered. For example, adenosine triphosphate is a mixture of three species (ATP^{4-}, $HATP^{3-}$, and H_2ATP^{2-}) in the middle pH range, and dissolved carbon dioxide is a mixture of four species (CO_2(aq), H_2CO_3, HCO_3^-, and CO_3^{2-}). It becomes much easier to handle these mixtures if the pH is specified, because the ratios between the equilibrium concentrations of the various species are then fixed and thermodynamic properties of the entities ATP and CO_2tot (i.e., CO_2(aq), H_2CO_3, HCO_3^-, and CO_3^{2-}) can be calculated.

A practical reason for the use of the apparent equilibrium constant K' is that most methods of measurement are unable to distinguish between the various biochemical species and, in fact, it is sums of species that are almost always measured. This makes the apparent equilibrium constant a convenient quantity for reporting the results of equilibrium measurements.

Before proceeding further, it is necessary to introduce some chemical equilibrium properties more formally than has been done to this point. Chemical thermodynamics is based primarily on the Gibbs energy G, which is a function of temperature, pressure, and amounts of species. This is the thermodynamic potential that provides the criterion for spontaneous change and equilibrium when the independent variables are temperature, pressure and amounts of species. Chemical thermodynamics can be said to be primarily based on G because the equilibrium constant K for a chemical reaction is given by $K = \exp(-\Delta_rG°/RT)$, where $\Delta_rG°$ is the standard reaction Gibbs energy. If K is determined over a range of temperature, the change in entropy in a chemical reaction $\Delta_rS°$ can be calculated using

$$\Delta_rS° = -\left(\frac{\partial \Delta_rG°}{\partial T}\right)_P. \qquad (2-1)$$

The change in enthalpy in a chemical reaction $\Delta_rH°$ can be calculated using

$$\Delta_rH° = -T^2\left(\frac{\partial(\Delta_rG°/T)}{\partial T}\right)_P. \qquad (2-2)$$

If hydrogen ions are required to balance a chemical reaction, the concentration of H^+ is included in the expression for the chemical equilibrium constant. Thus, the hydrolysis of ATP^{4-} is described by means of a chemical equation such as

$$ATP^{4-} + H_2O = ADP^{3-} + HPO_4^{2-} + H^+ \qquad (2-3)$$

A chemical equation balances atoms of elements and charge. However, if the pH is specified, the corresponding biochemical equation does not balance hydrogens and, therefore, does not balance electric charge. Chemical Eq. (2-3) leads to the following expression for the chemical equilibrium constant.

$$K = \frac{\left[ADP^{3-}\right]\left[HPO_4^{2-}\right]\left[H^+\right]}{\left[ATP^{4-}\right](c°)^2}. \qquad (2-4)$$

Chemical reactions like this are often referred to as reference reactions (see Section 7). It should be noted that the choice of the reference reaction (2-3) was arbitrary, and that several other reference reactions could have been selected in its place. Also, the standard concentration $c° = 1$ mol L^{-1} is included in the above equation in order to keep the equilibrium constant dimensionless.

When biochemical reactions are considered at specified pH they should be written in a form that does not explicitly show charges, because charges cannot be balanced if the pH is fixed:

$$ATP + H_2O = ADP + \text{phosphate} \qquad (2-5)$$

Similar considerations apply to fixed pMg: for example, in Eq. (2-5) ATP refers to the equilibrium mixture of ATP^{4-}, $HATP^{3-}$, H_2ATP^{2-}, $MgATP^{2-}$, $MgHATP^-$, and Mg_2ATP at the specified pH and pMg. The expression for the apparent equilibrium constant is given by

$$K' = \frac{[ADP][\text{phosphate}]}{[ATP]c°} \qquad (2-6)$$

Expressions for apparent equilibrium constants K' are written in terms of sums of species, rather than species, and apparent equilibrium constant K' of enzyme-catalyzed reactions usually vary with the pH. The qualification "apparent" emphasizes this difference from chemical thermodynamics. There are several reasons why $[H^+]$ cannot be included in the expression for an apparent equilibrium constant. The first is that $[H^+]$ is defined by the specified pH, and cannot then be calculated from the value of the apparent equilibrium constant, as in chemical thermodynamics; in other words, chemical thermodynamics treats $[H^+]$ as a dependent variable, whereas biochemical thermodynamics treats it as an independent variable, because the investigator chooses the pH. A second reason is that if the pH is specified, it is in principle held constant during the reaction, this being normally achieved, at least approximately, by the use of a suitable buffer, or more exactly with a pH-stat, a device that holds the pH constant by adding acid or alkali. It follows that hydrogen atoms are not conserved in a biochemical reaction system like atoms of other elements. As a buffer will allow some change in pH as the system

approaches equilibrium, the pH should be measured at equilibrium, because that is the pH to which the apparent equilibrium constant refers to. When an apparent equilibrium constant K' is used, it is essential to specify the biochemical reaction that it refers to, and to include all units, and other relevant conditions, always T, P, pH, and ionic strength I, and often pMg or pCa as well. The quantity pH is conveniently measured with a pH electrode. However, the quantities pMg and pCa, in the absence of electrodes of sufficient accuracy, are generally calculated from a knowledge of the concentrations of the solutes and the required metal-ion binding constants.

The apparent equilibrium constant is related to the standard transformed Gibbs energy of reaction $\Delta_r G'^\circ$ by

$$\Delta_r G'^\circ = -RT\ln K'. \qquad (2-7)$$

The property $\Delta_r G'^\circ$ (not $\Delta_r G^\circ$) has to be used when the pH is specified because it is a function of the transformed Gibbs energy of formation $\Delta_f G'$ that is discussed later (Section 5). The ordinary Gibbs energy G does not provide the criterion for spontaneous change and equilibrium when the independent variables (variables set by the investigator) include variables (typically pH) in addition to T and P. The subscript r refers to a reaction and is not necessary, but it is useful in distinguishing the standard transformed Gibbs energy of reaction $\Delta_r G'^\circ$ from the standard transformed Gibbs energy of formation $\Delta_f G'^\circ(i)$ of reactant i.

Although the apparent equilibrium constants and thermodynamic properties serve many purposes in biochemistry, they cannot replace the chemical functions entirely, because these remain necessary for studying changes that occur when the pH (or pMg, etc.) changes: acid dissociations and dissociations of complex ions need to be represented by chemical equations, and mechanisms including pH effects involve chemical equilibrium constants.

Effects of ionic strength are very important in biochemical thermodynamics, and so the ionic strength should always be specified in reporting experimental results. In the extended Debye–Hückel equation [5], the ionic strength effects are proportional to the squared electric charge of an ion and so the effect of ionic strength on the thermodynamic properties of ATP^{4-} are 16 times greater than for a chloride ion. The role of ionic strength in biochemical thermodynamics is very different from the effect of pH because ionic strength affects the properties of the solvent, in the same sense that adding an inert organic liquid changes the properties of the solvent.

Because of these differences between chemical thermodynamics and biochemical thermodynamics, it is necessary to use a Legendre transform (see Eqs. (3-1) and (3-2) and Section 5) to define a new thermodynamic potential, the transformed Gibbs energy G', as shown later in Eq. (6-1), that provides the criterion for spontaneous change and equilibrium when the pH is specified. The transformed Gibbs energy involving the pH was introduced in four papers in 1992 [6–9]. More complete discussions of the uses of transformed thermodynamic properties are available in two books [10,11].

There is another significant difference between chemical thermodynamics and biochemical thermodynamics. Enzyme-catalyzed reactions conserve atoms of elements other than hydrogen, but some enzyme-catalyzed reactions conserve groups of atoms in addition. For example, the enzyme glutamate–ammonia ligase (EC 6.3.1.2) [12] catalyzes a reaction [13] with net effect as follows:

$$\text{ATP} + \text{glutamate} + \text{ammonia} = \text{ADP} + \text{phosphate} + \text{glutamine} \qquad (2-8)$$

However, although this process can be regarded as the sum of two simpler reactions:

$$\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{phosphate} \qquad (2-9)$$

$$\text{glutamate} + \text{ammonia} = \text{H}_2\text{O} + \text{glutamine} \qquad (2-10)$$

glutamate–ammonia ligase does not catalyze these reactions separately, though there are other enzymes (EC 3.6.1.3 and EC 3.5.1.2 respectively) that do. The complete reaction catalyzed by glutamate–ammonia ligase conserves not only C, O, N, and P atoms, but also an additional component, components being the entities that are conserved in a reaction (in studies of metabolic systems the term *moiety* is sometimes used with the same meaning). To understand the importance of components, consider the calculation of the equilibrium composition for the glutamate–ammonia ligase reaction: the expression for the apparent equilibrium constant and conservation equations for C, O, N, and P provide five mathematical relationships between the concentrations at equilibrium, but five equations cannot contain enough information to allow calculation of the six independent concentrations of the six reactants shown in Eq. (2-8). There must, therefore, be an additional conservation relationship, and the use of linear algebra (see Section 8) allows this to be identified, so that the equilibrium composition can be calculated.

Some enzyme-catalyzed reactions involve three additional components, and, in metabolic systems that include numerous reactions, there may be more. For example, a model of glycolysis in the parasite *Trypanosoma brucei* required four constraints, three of them obvious (conservation of adenine nucleotides, etc.), but the fourth was not at all obvious and involved 12 different reactants distributed between two compartments [14]. In such cases, the principles of the linear algebra are unchanged from those for single reactions, but their application is only feasible if carried out by computer [15].

3. Basic thermodynamics

The first law of thermodynamics introduces the internal energy U and states that the change in U in a homogeneous system is given by $\Delta U = q + w$, where q is the heat flow into the system, and w is the work done on the system. The enthalpy H is defined by

$$H = U + PV. \qquad (3-1)$$

The second law of thermodynamics introduces the entropy S and has two parts. The first part states that dq/T is independent of pathway for a reversible process, where d indicates a differential (an infinitesimal change). This makes it possible to define entropy S by $dS = dq_{rev}/T$, where the subscript rev implies a relationship that is valid only for a reversible process. The second part states that when a change of an isolated system from one state to another takes place spontaneously, ΔS is greater than zero. This provides a way to predict when a change in state can take place spontaneously, based on calorimetric measurements. However, the second part of the second law cannot be used directly to predict whether a chemical reaction will occur spontaneously at specified temperature and pressure, because this depends on additional kinetic and mechanistic considerations that are beyond the scope of thermodynamics.

The third law of thermodynamics states that the entropy of each pure element or substance in a perfect crystalline form is zero at absolute zero. This provides a way to determine the entropy of a crystalline substance by making calorimetric measurements. The use of entropies determined in this way has allowed for the determination of the entropies of a substantial number of crystalline biochemical substances. These entropies can then be combined with standard enthalpies of combustion, solubilities, and enthalpies of solution to calculate the formation properties of biochemical species in aqueous media. This method has been used [16] to obtain the standard formation properties of adenosine and the ATP, ADP, and AMP series in aqueous media.

Each of the three properties U, S, and H provides a criterion for spontaneous change and equilibrium in chemical thermodynamics. These criteria are summarized by $(dU)_{S,V} \leq 0$, $(dS)_{U,V} \geq 0$, and $(dH)_{S,P} \leq 0$, where the subscripts indicate the properties that are held constant.

However, none of them applies to changes at constant T and P, and to remedy this omission, Gibbs defined the thermodynamic property that we now call the Gibbs energy (often called the Gibbs free energy) G by the use of

$$G = H - TS. \tag{3-2}$$

Eqs. (3-1) and (3-2) are referred to as Legendre transforms [17], and each defines a new thermodynamic property by subtracting a product of intensive and extensive properties from a previously defined thermodynamic property. The pairs of properties are referred to as conjugate properties, and their products have the dimensions of energy, for example, TS in Eq. (3-2) has the dimensions of energy. Note that the conjugate property to V is $-P$, which accounts for the plus sign in Eq. (3-1). By subtracting TS from H (see reaction 3-2) one obtains a criterion for spontaneous change and equilibrium at specified T and P: $(dG)_{T,P} \leq 0$. The thermodynamics of systems of chemical reactions at specified T and P is based on the use of G.

We are also indebted to Gibbs for the introduction of the chemical potential μ_j of species j. The relations between the various thermodynamic properties of a chemical reaction system are obtained from the fundamental equation for G that shows how changes in the independent variables T, P, and n_j (amounts of chemical species) determine the differential of the Gibbs energy. The fundamental equation for the Gibbs energy is given by

$$dG = -SdT + VdP + \sum_{j=1}^{N} \mu_j dn_j = -SdT + VdP + \sum_{j=1}^{N} \Delta_f G_j dn_j, \tag{3-3}$$

where the differential of the amount of species j is dn_j and N is the number of different kinds of species in the system. When making calculations in chemical thermodynamics, μ_j can be replaced with $\Delta_f G_j$, the Gibbs energy of formation of species j. The independent variables for a system without reactions are T, P, and n_1, n_2, …, n_N; these are referred to as the natural variables for the Gibbs energy. Integration of the fundamental equation for G at constant values of the intensive variables yields

$$G = \sum_{j=1}^{N} \mu_j n_j = \sum_{j=1}^{N} \Delta_f G_j n_j. \tag{3-4}$$

The fundamental equation shows how S, V, and $\Delta_f G_j$ can be calculated. For example, $\Delta_f G_j = (\partial G/\partial n_j)_{T,P}$ when the amounts of other species are held constant.

4. Thermodynamics of chemical reactions

The effects of ionic strength on thermodynamic properties of species in aqueous solution are handled in different ways in chemical thermodynamics and in biochemical thermodynamics, and so ionic strength effects in chemical reactions are discussed first. When a chemical reaction occurs in a closed system, the changes in the amounts n_j of species depend on the stoichiometric numbers ν_j in the balanced chemical equation (ν is the Greek letter nu):

$$\sum_{j=1}^{N} \nu_j B_j = 0, \tag{4-1}$$

where B_j represents species j, and the ν_j values are positive for products and negative for reactants. The amount n_j of species j at any stage in a chemical reaction is given by

$$n_j = n_{j0} + \nu_j \xi, \tag{4-2}$$

where n_{j0} is the initial amount of species j in the system and ξ is the extent of reaction (ξ is the Greek letter xi). It is evident from this definition that ξ is an extensive property with the unit mole. The differential of the amount of species j is given by

$$dn_j = \nu_j d\xi. \tag{4-3}$$

When a single chemical reaction occurs in a closed system, substitution of Eq. (4-3) in Eq. (3-33--3) yields

$$dG = -SdT + VdP + \left(\sum_{j=1}^{N} \nu_j \Delta_f G_j\right) d\xi = -SdT + VdP + \Delta_r G d\xi, \tag{4-4}$$

where $\Delta_r G$ is the reaction Gibbs energy given by

$$\Delta_r G = \left(\frac{\partial G}{\partial \xi}\right)_{T,P} = \sum_{j=1}^{N} \nu_j \Delta_f G_j. \tag{4-5}$$

At equilibrium, the Gibbs energy of the system is at a minimum with $(\partial G/\partial \xi)_{T,P} = 0$. At the minimum Gibbs energy, the equilibrium condition is

$$\sum_{j=1}^{N} \nu_j \Delta_f G_{jeq} = 0, \tag{4-6}$$

where $\Delta_f G_{jeq}$ is the Gibbs energy of formation of species j at equilibrium. Notice that this relation has the same form as the chemical equation (Eq. (4-1)).

The Gibbs energy of formation $\Delta_f G_j$ of a species in aqueous solution depends on the concentration of the species according to

$$\Delta_f G_j = \Delta_f G_j^\circ + RT\ln(\gamma_j [B_j]), \tag{4-7}$$

where $\Delta_f G_j^\circ$ is the standard Gibbs energy of formation of species j, γ_j is the activity coefficient of species j, and $[B_j]$ is its concentration. Activity coefficients are dimensionless, and so the logarithmic term should be divided by c°, which is the standard concentration (1 M), so that the logarithm is taken of a dimensionless quantity. But the c° is omitted as a simplification. In dilute aqueous solutions, γ_j is equal to unity for uncharged species, but γ_j of an ion depends on its electric charge, the ionic strength, and temperature. When activity coefficients are used in this way, $\Delta_f G_j^\circ$, $\Delta_f H_j^\circ$, and K are independent of ionic strength.

In biochemical thermodynamics, it is more practical to take $\Delta_f G_j^\circ$, $\Delta_f H_j^\circ$, and K to be a function of ionic strength when dealing with species. Eq. (4-7) can be written as

$$\Delta_f G_j = \Delta_f G_j^\circ + RT\ln\gamma_j + RT\ln[B_j]. \tag{4-8}$$

In biochemical thermodynamics, this equation is written as

$$\Delta_f G_j = \Delta_f G_j^\circ + RT\ln[B_j], \tag{4-9}$$

where the standard Gibbs energy of formation $\Delta_f G_j^\circ$ is a functions of ionic strength as well as temperature. This is useful because, when chemical equilibrium constants K are needed in biochemical thermodynamics (for example, K_a and K_{ref}), they are expressed in terms of the concentrations of species without activity coefficients. When this is done, K is a function of the ionic strength. Substituting Eq. (4-9) at equilibrium in Eq. (4-6) yields

$$\sum_{j=1}^{N} \nu_j \Delta_f G_j^\circ = -RT \sum_{j=1}^{N} \nu_j \ln[B_j]_{eq} = -RT \ln \prod_{j=1}^{N} [B]_{eq}^{\nu_j} = -RT\ln K, \tag{4-10}$$

where K is

$$K = \prod_{j=1}^{N} [B_j]_{eq}^{\nu_j}. \quad (4-11)$$

This equation is often used without the subscripts eq that indicates that the concentrations are equilibrium values. Eq. (4-10) shows that the standard reaction Gibbs energy $\Delta_r G^\circ$ is given by

$$\Delta_r G^\circ = \sum_{j=1}^{N} \nu_j \Delta_f G_j^\circ = -RT \ln K. \quad (4-12)$$

Substituting Eq. (4-9) in Eq. (4-5) yields

$$\Delta_r G = \Delta_r G^\circ + RT \ln Q, \quad (4-13)$$

where the reaction quotient Q has the form of the expression for the equilibrium constant, but with arbitrary concentrations of species.

In calculating the equilibrium composition for a chemical reaction system, it is important to understand the concept of components (see end of Section 2). Here, we use the chemical definition of components as the substances of fixed composition (i.e., pure chemical compounds) that comprise a mixture. The number of components C in a mixture is the minimum number of these chemical substances that are needed to prepare this mixture in all of the phases in which it exists. Of course the atoms of all elements are conserved in chemical thermodynamics, but the number of components is often less than the number of elements because only independent conservation equations can be used to calculate the equilibrium composition. Various choices can be made of components, but the number of components C is independent of the choice. In a multi-reaction system, there are also various sets of chemical reactions that can be used to calculate the equilibrium composition, but the number R of independent reactions is independent of the choice. When a chemical reaction system contains N species, $N = C + R$.

The value of a chemical equilibrium constant must always be accompanied by a chemical equation, the direction of the reaction to which the equilibrium constant refers, and, when Eq. (4-9) is used in aqueous solutions (as it is in biochemical thermodynamics), the ionic strength must be specified in addition to the temperature. Each species in a chemical reaction contributes its $\Delta_f G_j^\circ$ to the standard reaction Gibbs energy $\Delta_r G^\circ$ and to the equilibrium constant; this makes it possible to construct tables of standard thermodynamic properties of species at zero ionic strength. Measurements of K over a range of temperatures make it possible to calculate $\Delta_f G_j^\circ$, $\Delta_f H_j^\circ$, and $\Delta_f S_j^\circ$ (or S_j°) for species. Calorimetric measurements of enthalpies of reaction make it possible to calculate $\Delta_f H_j^\circ$, and calorimetric measurements on crystals down to close to absolute zero make it possible to obtain standard molar entropies of crystalline substances. These properties of species are given in the NBS Tables [4] and CODATA Tables [18] at 298.15 K (25 °C) and zero ionic strength. IUPAC's *Quantities, Units and Symbols for Physical Chemistry*, 3rd ed. [19] includes symbols for chemical thermodynamics.

5. Legendre transform to introduce the pH as an independent variable in biochemical thermodynamics

In his book on thermodynamics from the viewpoint of a physicist, Callen [20] writes "the choice of variables in terms of which a given problem is formulated, while a seemingly innocuous step, is often the most crucial step in the solution." He is referring to independent variables, i.e., variables under the control of the investigator. Specifying the pH may seem to be an innocuous step, but it has major effects in biochemical thermodynamics. The independent variables for a thermodynamic system determine the equilibrium state that is finally reached. Intensive independent variables have extensive conjugates, and extensive independent variables have intensive conjugates. Since the equilibrium compositions reached by most enzyme-catalyzed reactions are affected by the pH, it is necessary to define a new thermodynamic property, the transformed Gibbs energy G' by use of a Legendre transform (see first part of Section 3) in which a product of conjugate variables $n_c(H)\mu(H^+)$ is subtracted from G [6,7]. The amount of the hydrogen component $n_c(H)$ is the total amount of hydrogen atoms in the chemical reaction system that is given by $n_c(H) = \sum N_H(j) n_j$, where $N_H(j)$ is the number of hydrogen atoms in species j. The Legendre transform that defines the transformed Gibbs energy G' is

$$G' = G - n_c(H)\mu(H^+) = G - n_c(H)\left\{\Delta_f G^\circ(H^+) - RT \ln(10) \mathrm{pH}\right\}. \quad (5-1)$$

The second form of this equation shows how the chemical potential of H^+(aq) is related to the pH. (Note: hydron is the general name for the cation H^+ without regard to isotopic state.) The standard Gibbs energy of formation of H^+(aq), $\Delta_f G^\circ(H^+)$, is equal to zero at zero ionic strength by definition, but it is not equal to zero at higher ionic strengths. The pH in Eq. (5-1) is defined as $-\log_{10}[H^+]$, rather than as $-\log_{10}\{\gamma(H^+)[H^+]\}$. At 298.15 K, the difference $(\mathrm{pH} - \log_{10}\{\gamma(H^+)[H^+]\}) = -0.11$ at 0.10 mol L^{-1} ionic strength and -0.14 at 0.25 mol L^{-1} ionic strength [21]. Logarithms can only be calculated for dimensionless quantities. Using a Legendre transform is the only way the pH can be made an independent variable.

The fundamental equation for the transformed Gibbs energy is

$$dG' = -S'dT + VdP + \sum_{j=1}^{N-1} \mu_j' dn_j + RT \ln(10) n_c(H) d\mathrm{pH}, \quad (5-2)$$

where $S' = S - n_c(H)S(H^+)$. (This is the Legendre transform defining the transformed entropy at specified pH. There is a corresponding Legendre transform defining the transformed enthalpy H' at specified pH.) The transformed chemical potential of species j is given by

$$\mu_j' = \mu_j - N_H(j)\mu(H^+). \quad (5-3)$$

Note that the term for H^+ in the summation in Eq. (5-2) is equal to zero because $\mu(H^+) - N_H(H^+)\mu(H^+) = 0$. The Legendre transform has replaced the term $\mu(H^+)dn(H^+)$ in the summation with a new type of term, $RT\ln(10)n_c(H)d\mathrm{pH}$, where $n_c(H)$ is the amount of hydrogen atoms in the system. Then,

$$dG' = -S'dT + VdP + \sum_{j=1}^{N-1} \Delta_f G_j' dn_j + RT \ln(10) n_c(H) d\mathrm{pH}. \quad (5-4)$$

Here, μ_j' has been replaced by $\Delta_f G_j'$ to permit numerical calculations to be made.

The summation in Eq. (5-4) has a term for each species, except for H^+, but $\Delta_f G_j'$ has the same value for ATP^{4-}, $HATP^{3-}$, and H_2ATP^{2-}, for example [11]. Thus Eq. (5-4) can be written, as follows, in terms of amounts of reactants (sums of species like the three species of ATP) n_i' and the transformed Gibbs energy of formation of the reactants $\Delta_f G_i'$:

$$dG' = -S'dT + VdP + \sum_{i=1}^{N'} \Delta_f G_i' dn_i' + RT \ln(10) n_c(H) d\mathrm{pH}. \quad (5-5)$$

The species have been represented by j, and so the reactants (sums of species) are represented by i. There are N' different reactants. The natural variables for the transformed Gibbs energy are T, P, pH, and n_1', n_2', …, $n_{N'}'$. This reduction in the number of natural variables for G' is a result of the specification of the pH. The transformed Gibbs energy provides the following criterion for spontaneous change and equilibrium: $(dG') \le 0$, where the temperature, pressure, pH, ionic strength, and amounts of N' reactants are specified. Fundamental Eq. (5-5) shows how S', V, $\Delta_f G_i'$, and $RT\ln(10)n_c(H)$ can be calculated from G'. Note that $n_c(H) = \Sigma n_i' \overline{N}_H(i)$. The average number of hydrogen atoms in a reactant

is represented by $\overline{N}_H(i)$. Eq. (5-5) shows that $\Delta_f G_i' = (\partial G'/\partial n_i') = \Delta_f G_i'^\circ + RT\ln[B_i]$ at constant T, P, pH and amounts of other reactants.

It is the Maxwell relations [21,22], which are mixed partial derivatives, that yield useful relations between the various thermodynamic properties. If the VdP term is ignored and the transformed enthalpy H' is introduced using $G' = H' - TS'$, there are five Maxwell relations. They are written here in terms of standard formation properties for reactants because the concentration terms cancel on the two sides of the equations:

$$-\Delta_f S_i'^\circ = \frac{\partial \Delta_f G_i'^\circ}{\partial T} \quad (5-6)$$

$$-\frac{\partial \Delta_f S_i'^\circ}{\partial T} = RT\ln(10)\frac{\partial (T\overline{N}_H(i))}{\partial T} \quad (5-7)$$

$$\frac{\partial \Delta_f G_i'^\circ}{\partial \mathrm{pH}} = RT\ln(10)\overline{N}_H(i) \quad (5-8)$$

$$\Delta_f H_i'^\circ = -T^2\frac{\partial\left(\Delta_f G_i'^\circ / T\right)}{\partial T} \quad (5-9)$$

$$\frac{\partial \Delta_f H_i'^\circ}{\partial \mathrm{pH}} = -RT^2\ln(10)\frac{\partial \overline{N}_H(i)}{\partial T}. \quad (5-10)$$

Note that ln(10) is approximately 2.303. These equations are important because they show that when $\Delta_f G_i'^\circ$ can be expressed as a function of T, pH, and ionic strength based on experimental data, all the other thermodynamic properties of a biochemical reactant can be obtained by taking partial derivatives. Note that there are alternative routes to $(\partial \Delta_f S'^\circ/\partial \mathrm{pH})$ and $(\partial \Delta_f H'^\circ/\partial \mathrm{pH})$ because they can be calculated directly or they can be calculated from the function for $\overline{N}_H(i)$.

6. Equations for the standard transformed formation properties of a reactant

Eq. (5-3) shows that the species that make up a reactant have transformed Gibbs energies of formation $\Delta_f G_j'$ as well as Gibbs energies of formation $\Delta_f G_j$.

$$\Delta_f G_j' = \Delta_f G_j - N_H(j)\Delta_f G(H^+) \quad (6-1)$$

Substituting $\Delta_f G_j = \Delta_f G_j^\circ + RT\ln[B_j]$ and $\Delta_f G[H^+] = \Delta_f G^\circ[H^+] + RT\ln(10^{-\mathrm{pH}})$ into Eq. (6-1) yields

$$\Delta_f G_j' = \Delta_f G_j^\circ + RT\ln[B_j] - N_H(j)\left\{\Delta_f G^\circ[H^+] - RT\ln(10)\mathrm{pH}\right\}$$
$$= \Delta_f G_j'^\circ + RT\ln[B_j], \quad (6-2)$$

where the standard transformed Gibbs energy of formation of species j is given by

$$\Delta_f G_j'^\circ = \Delta_f G_j^\circ - N_H(j)\left\{\Delta_f G^\circ[H^+] - RT\ln(10)\mathrm{pH}\right\}. \quad (6-3)$$

The standard Gibbs energies of formation $\Delta_f G_j^\circ$ and $\Delta_f G^\circ(H^+)$ depend on the ionic strength I. The standard Gibbs energy of formation of species j at ionic strength I can be estimated using the extended Debye–Hückel equation [5].

$$\Delta_f G_j^\circ(I) = \Delta_f G_j^\circ(I=0) - \alpha z_j^2 I^{1/2}/\left(1 + 1.6I^{1/2}\right), \quad (6-4)$$

where z_j is the charge on species j, and α is the parameter in the Debye-Hückel equation that can be represented by a power series in the temperature [23]. The 1.6 $\mathrm{kg}^{1/2}\,\mathrm{mol}^{-1/2}$ is an empirical parameter that is assumed to be independent of temperature. When Eq. (6-4) is substituted for $\Delta_f G_j^\circ$ and $\Delta_f G^\circ[H^+]$ in Eq. (6-3), the following equation yields the standard transformed Gibbs energy of species j at a specified ionic strength:

$$\Delta_f G_j'^\circ(I) = \Delta_f G_j^\circ(I=0) - N_H(j)RT\ln(10)\mathrm{pH} - \alpha\left(z_j^2 - N_H(j)\right)I^{1/2}$$
$$/\left(1 + 1.6I^{1/2}\right). \quad (6-5)$$

This shows that $\Delta_f G_j'^\circ(I)$ is a function of ionic strength for uncharged species that contain hydrogen atoms, as well as charged species. There is an exception to this statement when $z_j^2 - N_H(j) = 0$ for a species. The standard transformed Gibbs energy of formation of a species is independent of ionic strength and pH when $z_j = 0$ and $N_H(j) = 0$. Eq. (6-5) shows how the standard transformed Gibbs energy of formation of a biochemical reactant consisting of a single species is calculated from the standard Gibbs energy of formation of the species at zero ionic strength.

When there are two or more species in a pseudoisomer group (like ATP^{4-}, $HATP^{3-}$, and H_2ATP^{2-} at specified pH), the standard transformed Gibbs energy of formation $\Delta_f G_i'^\circ$ of the pseudoisomer group is calculated using [24]

$$\Delta_f G_i'^\circ = -RT\ln\left\{\sum_{j=1}^{N_{iso}}\exp\left[-\Delta_f G_j'^\circ/RT\right]\right\}, \quad (6-6)$$

where N_{iso} is the number of species in the pseudoisomer group.

The standard transformed enthalpy of reactant i is given by

$$\Delta_f H'^\circ = \sum_{j=1}^{N_{iso}} r_j \Delta_f H_j'^\circ, \quad (6-7)$$

where r_j is the equilibrium mole fraction of species j that is given by

$$r_j = \exp\left\{\left[\Delta_f G'^\circ(\mathrm{reactant}) - \Delta_f G_j'^\circ\right]/(RT)\right\}. \quad (6-8)$$

These calculations are sufficiently complicated that a computer is required, as well as software with symbolic and calculus capabilities [11,25]. Mathematical applications for computers can be used to derive the function of pH, and ionic strength that yields the standard transformed Gibbs energy $\Delta_f G_i'^\circ$ of a reactant at 298.15 K by combining Eqs. (6-5) and (6-6). The function for $\Delta_f G_i'^\circ$ is readily evaluated for desired pHs, and ionic strengths. The average number of hydrogen atoms $\overline{N}_H(i)$ in the reactant at 298.15 K at the specified pH and ionic strength can be calculated using Eq. (6-8). This property can also be calculated using

$$\overline{N}_H(i) = \sum_{j=1}^{N} r_j N_{H(j)} \quad (6-9)$$

where N is the number of different species and r_j is the equilibrium mole fraction of the jth species in the reactant that can be calculated using the binding polynomial [26–28], which utilizes the pKs of species.

When the standard enthalpies of formation $\Delta_f H_j^\circ$ at zero ionic strength are known at 298.15 K for the species of a reactant, the $\Delta_f G_j^\circ(T, I=0)$ can be calculated over a range of temperatures using [28,29]

$$\Delta_f G_j^\circ(T, I=0) = \frac{T}{298.15\,\mathrm{K}}\Delta_f G_j^\circ(298.15\,\mathrm{K}, I=0)$$
$$+\left(1 - \frac{T}{298.15\,\mathrm{K}}\right)\Delta_f H_j^\circ(298.15\,\mathrm{K}, I=0). \quad (6\text{–}10)$$

This is based on the assumption that $\Delta_f H_j^\circ$ is independent of temperature over a modest temperature range, e.g. 273.15 K to 313.15 K. Other properties at specified temperature, pH, and ionic strength can be calculated by the use of Eqs. (5-6) to (5-10).

Tables of transformed thermodynamic properties of reactants can be produced, but this does not fully satisfy the needs of biochemists. It is more useful to have mathematical functions in a computer that give these properties so that they can be calculated at desired temperatures, pHs, and ionic strengths (see Section 10).

7. Thermodynamics of biochemical reactions

When a biochemical reaction occurs alone, the changes in the amounts n_i' of reactants depend on the stoichiometric numbers ν_i' in the balanced biochemical reaction equation. The balanced biochemical equation is represented by

$$\sum_{i=1}^{N'} \nu_i' B_i = 0, \tag{7-1}$$

where B_i is a sum of species and N' is the number of different reactants. Biochemical reactions balance the atoms of all elements except for hydrogen when they are carried out at a specified pH. (If pMg is held constant, the biochemical equation does not balance magnesium atoms.) The stoichiometric numbers ν_i' are positive for products and negative for reactants. The amount n_i' of reactant i at any stage in a biochemical reaction is given by

$$n_i' = n_{i0}' + \nu_i' \xi', \tag{7-2}$$

where n_{i0}' is the initial amount of reactant i and ξ' is the extent of biochemical reaction. The differential of the amount of reactant i is given by

$$dn_i' = \nu_i' d\xi'. \tag{7-3}$$

When a single biochemical reaction occurs, the differential of the transformed Gibbs energy (see Eq. (5-5)) is given by

$$\begin{aligned} dG' &= -S'dT + VdP + \left(\sum_{i=1}^{N'} \nu_i' \Delta_f G_i'\right) d\xi' + RT\ln(10) n'(\mathrm{H}) d\mathrm{pH} \\ &= -S'dT + VdP + \Delta_r G' d\xi' + RT\ln(10) n'(\mathrm{H}) d\mathrm{pH}, \end{aligned} \tag{7-4}$$

where $\Delta_r G'$ is the transformed reaction Gibbs energy. The Maxwell equations of this fundamental equation are like Eqs. (5-6) to (5-10), except that the changes in properties apply to a biochemical reaction rather than a reactant, and $\overline{N}_H$ is replaced by $\Delta_r N_H$, the change in binding of H^+ in the biochemical reaction. The third and fourth terms in Eq. (7-4) show that the change in binding of H^+ in an enzyme-catalyzed reaction is given by [27]

$$\Delta_r N_H = \frac{1}{RT\ln(10)} \frac{\partial \Delta_r G'^\circ}{\partial \mathrm{pH}}. \tag{7-5}$$

This property of an enzyme-catalyzed reaction can also be calculated using [27,28]

$$\Delta_r N_H = \sum_{i=1}^{N'} \nu_i' \overline{N}_H(i), \tag{7-6}$$

where $\overline{N}_H(i)$ is given by Eq. (6-9).

The rate of change of G' with extent of reaction for a system having a single reaction at constant T, pH, and ionic strength is given by

$$\left(\frac{\partial G'}{\partial \xi'}\right)_{T,P,\mathrm{pH}} = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' = \Delta_r G' = \Delta_r G'^\circ + RT\ln Q' \tag{7-7}$$

where the biochemical reaction quotient Q' has the form of the apparent equilibrium constant, but with arbitrary concentrations of reactants. It is the sign of $\Delta_r G'$ that determines whether a biochemical reaction will go to the right or the left at specified reactant concentrations: a negative value indicates that the reaction can spontaneously go to the right.

The transformed Gibbs energy of the system is at a minimum at equilibrium, where $(\partial G'/\partial \xi')_{T,P,\mathrm{pH}} = 0$. At the minimum transformed Gibbs energy, the equilibrium condition is

$$\sum_{i=1}^{N'} \nu_i' \Delta_f G_{i\mathrm{eq}}' = 0. \tag{7-8}$$

Notice that this relation has the same form as the biochemical equation (Eq. (7-1)).

To calculate the apparent equilibrium constant for an enzyme-catalyzed reaction, the mathematical functions yielding $\Delta_f G_i'^\circ$ are added and subtracted according to

$$\Delta_r G'^\circ = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i'^\circ = -RT\ln K', \tag{7-9}$$

where the apparent equilibrium constant K' is given by

$$K' = \prod_{i=1}^{N'} [B_i]^{\nu_i}. \tag{7-10}$$

Whenever the value of an apparent equilibrium constant is given, it is necessary to show the way the biochemical reaction is written. It is possible to make tables with values of K' at specified temperatures, pHs, and ionic strengths, but it is much more useful to have mathematical functions for K' in a computer that can be evaluated at desired temperatures, pHs, and ionic strengths. Other thermodynamic properties of the enzyme-catalyzed reaction can be obtained by taking partial derivatives of $\Delta_r G'^\circ$.

Apparent equilibrium constants that depend on pH can be expressed by

$$K' = K_{\mathrm{ref}} 10^{n\mathrm{pH}} f(\mathrm{pH}), \tag{7-11}$$

where K_{ref} is a chemical equilibrium constant, n is the number of H^+ in the reference reaction (a positive integer if H^+ is produced and a negative integer if H^+ is consumed). The function $f(\mathrm{pH})$ brings in the pKs of the substrates. In thermodynamics, the choice of a reference reaction is arbitrary, and so n does not have a mechanistic significance, but this equation does suggest there are two types of pH effects — those involving pKs that extend over a couple of units of pH and effects of $10^{n\mathrm{pH}}$ that extend over the whole range of pH considered. The value of n can be determined from kinetic data.

This section has dealt with the pH as an independent variable, but that raises a question as to what other intensive variables can be introduced into biochemical thermodynamics. When the reaction system contains magnesium ions and magnesium complex ions are formed with one or more reactants, the following Legendre transform can be used to introduce $\mathrm{pMg} = -\log_{10}[Mg^{2+}]$ as an independent variable in addition to pH:

$$G' = G - n_c(\mathrm{H})\mu(H^+) + n_c(\mathrm{Mg})\mu(Mg^{2+}). \tag{7-12}$$

where $n_c(\mathrm{Mg})$ is the amount of the magnesium component, that is the total amount of magnesium atoms in the system. Since dissociation constants of magnesium complex ions are known for the ATP series, the effects of pMg on standard transformed thermodynamic properties in the ATP series have been calculated [30]. Since H^+ and Mg^{2+} compete, the effect of pH on the binding of Mg^{2+} by a reactant is equal to the effect of pMg on the binding of H^+:

$$\frac{\partial \overline{N}_{\mathrm{H}}}{\partial \mathrm{pMg}} = \frac{\partial \overline{N}_{\mathrm{Mg}}}{\partial \mathrm{pH}}, \tag{7-13}$$

where the first partial derivative is at constant pH and the second partial derivative is at constant pMg. These are called reciprocal effects.

When H_2O is a reactant in an enzyme-catalyzed reaction, there is a problem in calculating equilibrium compositions because $[H_2O]$ is omitted in the expression for the apparent equilibrium constant, but $\Delta_f G'^\circ(H_2O)$ is required in the calculation of the apparent equilibrium constant. This problem, which is discussed in the next section, can be solved by using a Legendre transform to define a further transformed Gibbs energy G'' using the following Legendre transform [31,32].

$$G'' = G' - n_c(\mathrm{O})\mu'^\circ(H_2O) = G' - n_c(\mathrm{O})\Delta_f G'^\circ(H_2O) \tag{7-14}$$

This is possible because, when H_2O is involved in an enzyme-catalyzed reaction, oxygen atoms are not conserved because an essentially infinite amount of oxygen atoms (in comparison with the amounts of reactants) is available from the solvent. This transformation leads to the further transformed Gibbs energy of formation $\Delta_f G''^\circ$ of a reactant. Since $\Delta_f G''^\circ(H_2O) = 0$, H_2O can be left out of the calculation of the standard transformed Gibbs energy of formation of an enzyme-catalyzed reaction.

$$\Delta_r G''^\circ = \sum_{i=0}^{N''} \nu_i'' \Delta_f G_i''^\circ = -RT \ln K'' \tag{7-15}$$

The ν_i'' are stoichiometric numbers when H_2O is omitted, and reactants are defined as pseudoisomer groups when oxygen atoms are not conserved.

In making calculations on systems of biochemical reactions, it may be of interest to specify the concentrations of coenzymes because they are involved in many reactions and may be in steady states. This provides a more global view of a system of biochemical reactions [33]. When the concentrations of coenzymes are specified, the criterion for spontaneous change and equilibrium is provided by the further transformed Gibbs energy defined by the following Legendre transform:

$$G'' = G' - \sum n_c(\mathrm{coenzyme})\{\Delta_r G'^\circ(\mathrm{coenzyme}) + RT \ln[\mathrm{coenzyme}]\}, \tag{7-16}$$

where n_c(coenzyme) is the amount of the coenzyme component. The identification of components is discussed in the next section. A reaction system has a finite number of components, and Legendre transforms can be used to specify all but one component. Applying Legendre transforms to all components in a system of reactions yields the Gibbs–Duhem equation that is a relation between the intensive properties of a system that is equal to zero. Thus, all but one of the intensive variables for a reaction system at equilibrium can be specified.

8. Stoichiometry

8.1. Stoichiometry of chemical reactions

The stoichiometry of chemical reactions is well understood, but it involves more than the fact that chemical reactions balance atoms of elements and electric charge. A chemical equation can be represented by a matrix multiplication, and this is of more than just theoretical interest, because it means that techniques developed in linear algebra for matrix manipulation can be applied directly to chemical equations. Consider the oxidation of methane to carbon dioxide and water in the gas phase, which can be written as

$$-CH_4 - 2O_2 + CO_2 + 2H_2O = 0 \tag{8-1}$$

Each species in the reaction can be represented by a column vector giving the number of carbon atoms, hydrogen atoms, and oxygen atoms:

$$-\begin{bmatrix}1\\4\\0\end{bmatrix} - 2\begin{bmatrix}0\\0\\2\end{bmatrix} + \begin{bmatrix}1\\0\\2\end{bmatrix} + 2\begin{bmatrix}0\\2\\1\end{bmatrix} = \begin{bmatrix}0\\0\\0\end{bmatrix} \tag{8-2}$$

This equation can be written as a matrix multiplication:

$$\begin{bmatrix}1&0&1&0\\4&0&0&2\\0&2&2&1\end{bmatrix}\begin{bmatrix}-1\\-2\\1\\2\end{bmatrix} = \begin{bmatrix}0\\0\\0\end{bmatrix} \tag{8-3}$$

The matrix on the left (a 3×4 matrix) is referred to as a conservation matrix ($\boldsymbol{A}$), and the 4×1 column vector is referred to as a stoichiometric number matrix (ν) because it gives the stoichiometric numbers in Eq. (8-1). This matrix multiplication is represented by

$$A\nu = \mathbf{0}, \tag{8-4}$$

where $\mathbf{0}$ is the corresponding zero matrix. This equation is useful because it makes it possible to calculate the stoichiometric number matrix from the conservation matrix. This operation is called calculating the null space of $\boldsymbol{A}$, and computer applications can be used to carry out this operation. It is necessary to say that this produces "a basis for the stoichiometric number matrix" because the stoichiometric number matrix is not unique; for example, multiplying a balanced chemical equation by an integer yields a balanced chemical equation.

Eq. (8-4) can also be written in terms of the transposed matrices.

$$\nu^{\mathrm{T}}\boldsymbol{A}^{\mathrm{T}} = \mathbf{0} \tag{8-5}$$

where T indicates the transpose. Calculating the null space of ν^{T} yields the transposed conservation matrix, or more precisely a basis for $\boldsymbol{A}^{\mathrm{T}}$.

The number of different species in a chemical reaction is represented by N, the number of different elements (that is, components) is represented by C, and the number of reactions is represented by R. Note that $N = C + R$. More precisely, the number of components C is the number of independent elements (that is, elements with conservation equations that do not differ by an integer factor), and R is the number of independent reactions. For a multireaction system, the conservation equation is $C\times N$ and the stoichiometric number matrix is $N\times R$, so that the zero matrix is $(C\times N)(N\times R) = C\times R$.

8.2. Stoichiometry of biochemical reactions

Biochemical equations can also be written as matrix multiplications. The hydrolysis of ATP to ADP at a specified pH can be written as

$$-\mathrm{ATP} - H_2O + \mathrm{ADP} + \mathrm{phosphate} = 0 \tag{8-6}$$

Each reactant is represented by a column vector giving the number of carbon atoms, oxygen atoms, nitrogen atoms, and phosphorus atoms:

$$-\begin{bmatrix}10\\13\\5\\3\end{bmatrix} - \begin{bmatrix}0\\1\\0\\0\end{bmatrix} + \begin{bmatrix}10\\10\\5\\2\end{bmatrix} + \begin{bmatrix}0\\4\\0\\1\end{bmatrix} = \begin{bmatrix}0\\0\\0\\0\end{bmatrix} \tag{8-7}$$

Hydrogen atoms are not conserved because the pH is specified. Row 3 can be deleted because it is a scalar product of row 1 and therefore provides no new information. Therefore, the nitrogen row is deleted to obtain

$$-\begin{bmatrix}10\\13\\3\end{bmatrix}-\begin{bmatrix}0\\1\\0\end{bmatrix}+\begin{bmatrix}10\\10\\2\end{bmatrix}+\begin{bmatrix}0\\4\\1\end{bmatrix}=\begin{bmatrix}0\\0\\0\end{bmatrix} \quad (8-8)$$

This biochemical equation can be written as a matrix multiplication.

$$\begin{bmatrix}10&0&10&0\\13&1&10&4\\3&0&2&1\end{bmatrix}\begin{bmatrix}-1\\-1\\1\\1\end{bmatrix}=\begin{bmatrix}0\\0\\0\\0\end{bmatrix} \quad (8-9)$$

This matrix multiplication is represented by

$$\boldsymbol{A}'\nu'=\mathbf{0} \quad (8-10)$$

The primes are needed to indicate that the reactions are written in terms of reactants (sums of species) at specified pH. Calculating the null space of $\boldsymbol{A}'$ yields ν'. Calculating the null space of $(\nu')^{\mathrm{T}}$ yields $(\boldsymbol{A}')^{\mathrm{T}}$.

$$(\nu')^{\mathrm{T}}(\boldsymbol{A}')^{\mathrm{T}}=\mathbf{0} \quad (8-11)$$

Note that the conservation matrix is $C'\times N'$, the stoichiometric number matrix is $N'\times R'$, and $N'=C'+R'$.

The number of components in an enzyme-catalyzed reaction is important because the conservation equations for components are constraints on the equilibrium that can be reached. As mentioned in Section 2, coupling by the enzyme mechanism increases the number of components beyond the number expected for the conservation of elements other than hydrogen. Thus, in enzyme-catalyzed reactions, the number of components can be larger than the number of different elements. In Eqs. (8-3) and (8-9), the elements are taken to be components, but the row reduction of $\boldsymbol{A}'$ shows that alternatively reactants can be taken as components. Row reduction of $\boldsymbol{A}'$ for ATP hydrolysis (see Eq. (8-9)) yields the following conservation matrix:

	ATP	H_2O	ADP	Phosphate
ATP	1	0	0	1
H_2O	0	1	0	1
ADP	0	0	1	−1

This shows that the components can be taken to be ATP, H_2O, and ADP, rather than C, O, and P. Coupling introduces components beyond the elements, and this is the way these additional components can be associated with reactants [33]. Fundamental equations and Maxwell equations can be written in matrix notation, which is useful in making calculations on systems of enzyme-catalyzed reactions [10].

One of the most basic calculations in biochemical thermodynamics is the calculation of the equilibrium composition. When this is done for chemical reactions or enzyme-catalyzed reactions, there is no analytic solution, and the equilibrium composition has to be calculated by use of an iterative method. Thermodynamic equilibrium calculations are discussed in the literature [10,11,25]. There are special problems when H_2O is a reactant because its "concentration" does not change.

9. Standard apparent reduction potentials for half reactions of enzyme-catalyzed reactions

Any oxidoreductase reaction can be written as the sum of two half reactions, that are independent of each other in the sense that the two electrochemical half reactions simply exchange formal electrons. In enzyme-catalyzed reactions these electrons are exchanged between half reactions through groups in the catalytic site. When the species properties of reactants in a half reaction have been determined, the standard apparent reduction potential E'° for the half reaction can be calculated over a range of pH. In writing half reactions for enzyme-catalyzed reactions, H^+ are omitted because it is understood that H^+ are supplied or neutralized to hold the pH at the specified value. (This is just a specific instance of the general policy of not including H^+ in the expressions for biochemical reactions.) Half reactions do balance atoms, except for hydrogen atoms, but they do not balance electric charge. If the $\Delta_f H^{\circ}$ of the species in all the reactants are known in addition to $\Delta_f G^{\circ}$, $\Delta_r G'^{\circ}$ can be calculated over a range of temperature as well as pH by using $E'^{\circ}=-\Delta_r G'^{\circ}/|\nu_e|F$. The number of electrons exchanged between the half reactions is $|\nu_e|$, and F is the Faraday constant (96 485.339 9 C mol^{-1}). Then the E'° for the half reaction can be calculated over a range of temperature as well as pH [34].

Tables of standard apparent reduction potentials are arranged in such a way that any oxidized reactant (sum of species) will react with the reduced reactant in a half reaction with a lower E'° when the reactant concentrations are all 1 M, except for H_2O. The "reactant" in this sentence can be a sum of reactants, like CO_2tot + pyruvate. The electrons in the two half reactions of an oxidoreductase reaction must cancel. The rule in making such a table is that there should be no fractional stoichiometric numbers, but half reactions can be multiplied by an integer or divided by an integer without changing the standard apparent reduction potential of the half reaction.

Half reactions are written by convention as reduction reactions, i.e. with the electrons appearing as reactants on the left-hand side of each half reaction. In aqueous solutions, standard apparent reduction potentials at pH 7 for half reactions of biochemical interest are largely restricted to the range 0.81 V to −0.42 V at 298.15 K, pH 7, and 0.25 mol L^{-1} ionic strength because half reactions with potentials higher than 0.81 V lead to the spontaneous production of O_2 gas and half reactions with potentials lower than −0.42 V lead to the spontaneous production of H_2 gas.

$$O_2(g)+4e=2H_2O \qquad E'^{\circ}=0.81\,V \quad (9-1)$$

$$2e=H_2(g) \qquad E'^{\circ}=-0.42\,V \quad (9-2)$$

In the hydrogen half reaction, H^+ is not shown on the left because it is understood that the half reaction occurs at a specified pH.

The standard transformed Gibbs energy of an oxidoreductase reaction is given by

$$\Delta_r G'^{\circ}=-|\nu_e|FE'^{\circ}=-|\nu_e|F(E'^{\circ}_h-E'^{\circ}_1)=\Delta_r G'^{\circ}_h-\Delta_r G'^{\circ}_1, \quad (9-3)$$

where E'° is the standard apparent electrode potential for the oxidoreductase reaction. E'°_h is the standard apparent reduction potential for the half reaction higher in the table, and E'°_l is the standard apparent reduction potential lower in the table. $\Delta_r G'^{\circ}_h$ and $\Delta_r G'^{\circ}_l$ are the standard transformed Gibbs energies of the two half reactions.

The apparent equilibrium constant for an oxidoreductase reaction is given by

$$K'=\exp(|\nu_e|FE'^{\circ}/RT). \quad (9-4)$$

If E'° can be calculated or measured over a range of pHs, the change in binding of H^+ in the oxidoreductase reaction can be calculated from the difference in $\partial E'^{\circ}/\partial$pH for the two half reactions. The change in binding of H^+ in the oxidoreductase reaction is equal to the difference between the change in binding in the higher half reaction $\Delta_r N_{Hh}$ and the change in binding in the lower half reaction $\Delta_r N_{Hl}$.

$$\Delta_r N_H=\Delta_r N_{Hh}-\Delta_r N_{Hl}=-\frac{|\nu_e|F}{RT\ln(10)}\left(\frac{\partial E'^{\circ}_h}{\partial \mathrm{pH}}-\frac{\partial E'^{\circ}_l}{\partial \mathrm{pH}}\right). \quad (9-5)$$

When K' is calculated or measured over a range of temperature, $\Delta_r G'^\circ$, $\Delta_r H'^\circ$, and $\Delta_r S'^\circ$ can be calculated for the oxidoreductase reaction and its half reactions [34].

Tables of standard apparent reduction potentials can be prepared at desired temperatures, pHs, and ionic strengths. If there are N half reactions in a table, E'° and K' can be calculated for $N(N-1)/2$ oxidoreductase reactions. The E'° table in *Biochemical Thermodynamics: Applications of Mathematica* [11] contains 60 half reactions, and so it can be used to calculate the apparent equilibrium constants for $60\times 59/2 = 1770$ oxidoreductase reactions. Enzymes are not known for all these reactions.

10. Thermodynamic tables

Since transformed thermodynamic properties at specified pH can all be traced back to thermodynamic properties of species, BasicBiochemData3 [11,35] gives $\Delta_f G_j^\circ$, $\Delta_f H_j^\circ$, z_j, and $N_H(j)$ for species of reactants in small matrices with a row for each species. The $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ are in kJ mol^{-1} at 298.15 K and zero ionic strength, as in the NBS Tables [4] and the CODATA Tables [18]. Small matrices are used because it is easy to extract any one of these properties or any combination of these properties.

BasicBiochemData3 [11,35] was started with inorganic species and organic species up to C_2 from the standard tables of chemical thermodynamic properties. Data on some species of biochemical interest were obtained from Wilhoit [36] and Thauer, Jungermann, and Decker [37]. These data made it possible to calculate $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ of more species from K' and $\Delta_r H(\text{cal})$ in the compilations by Goldberg and coworkers [38–45]. In order to obtain $\Delta_f G_j^\circ$ of the species of a reactant, it is necessary to have an experimental value of K' for an enzyme-catalyzed reaction where $\Delta_f G_j^\circ$ is already known for the species of all the reactants but one. If the reactant of interest has pK values that affect the relative concentrations of species in the range pH 5 to 9, it is necessary to know these values at 298.15 K and zero ionic strength. These are very demanding requirements, but as the database grows, it will be possible to use more of the data in the compilations of Goldberg and coworkers.

There is one exception to these requirements, which is illustrated by the ATP series. When Alberty and Goldberg [8] calculated $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ for the species in the ATP series, it was not possible to connect any one of these species with the elements, and so $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ for adenosine^0 in dilute aqueous solution were taken to be zero as a convention of the thermodynamic table. The $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ for the reactants in the ATP series calculated in this way can be used to calculate K' for reactions involving the reactants in the ATP series provided the adenosine moiety is on both sides of the biochemical equation. In 2001, Boerio-Goates and coworkers [16] determined the entropy of crystalline adenosine by the third-law method and with the enthalpy of adenosine from combustion calorimetry were able to calculate $\Delta_f G^\circ$(adenosine^0,aq,298.15 K) and $\Delta_f H^\circ$(adenosine^0,aq,298.15 K) with respect to the elements in their reference states. This made it possible to calculate $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ with respect to the elements for all the species in the ATP series. In BasicBiochemData3 [35], the convention that $\Delta_f G_j^\circ = 0$ and $\Delta_f H_j^\circ = 0$ has been used for a dozen additional species. These are mostly oxidized forms of reactants in oxidoreductase reactions. This convention is valid when the oxidized form is on one side of the reaction and the reduced form is on the other side of the reaction. The calculations of $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ from experimental values of K' and $\Delta_r H(\text{cal})$ are sufficiently complicated that computer programs are needed [11].

In the absence of experimental values for K' and $\Delta_r H(\text{cal})$ from which species properties can be calculated, estimates of $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ of species can be made by use of knowledge of these properties for similar reactants. A good example of this is the use of data on the ATP series to estimate $\Delta_f G_j^\circ$ and $\Delta_f H_j^\circ$ of species in the inosine triphosphate series by Boerio-Goates and coworkers [46]. They pointed out that "on the basis of structural similarity, one would expect the pK and $\Delta_f H_j^\circ$ values for the H^+(aq) and Mg^{2+}(aq) binding reactions involving the corresponding phosphate groups in the adenosine 5′-phosphate series and the inosine 5′-phosphate series to have essentially the same values." Structural similarity has also been used to estimate species properties of guanine, xanthine, and their nucleosides and nucleotides [47].

BasicBiochemData3 [35] provides data on the properties of chemical species of 199 biochemical reactants at 298.15 K. These species properties make it possible to calculate standard transformed Gibbs energies of formation, standard transformed enthalpies of formation, and standard transformed entropies of formation of these 199 reactants at specified pHs in the range of pH 5 to pH 9. This makes it possible to calculate apparent equilibrium constants for a large number of enzyme-catalyzed reactions.

11. Relations between biochemical thermodynamics and enzyme kinetics

The connection between biochemical thermodynamics and biochemical kinetics is provided by Haldane relations, which are expressions for the apparent equilibrium constant in terms of kinetic parameters in the steady-state rate equation. When the kinetic parameters for the reverse reaction can be determined in addition to those for the forward reaction, the numerator for the rate law for the catalyzed reaction contains a difference of terms that is equal to zero at equilibrium. At equilibrium, the rate in the forward rate direction is equal to that in the back direction. Therefore, a reaction that is at equilibrium proceeds at a rate of zero. The rate v of an enzyme-catalyzed reaction A = P may, in the simplest case, be given by a form of the Michaelis–Menten equation generalized to the reversible case:

$$v = \frac{V_f[\mathrm{A}]/K_\mathrm{A} - V_r[\mathrm{P}]/K_\mathrm{P}}{1 + [\mathrm{A}]/K_\mathrm{A} + [\mathrm{P}]/K_\mathrm{P}}, \qquad (11-1)$$

where V_f is the limiting velocity for the forward reaction, V_r is the limiting velocity for the reverse reaction, K_A is the Michaelis constant for A, and K_P is the Michaelis constant for P. The Haldane relation is

$$K' = \frac{V_f K_\mathrm{P}}{V_r K_\mathrm{A}}. \qquad (11-2)$$

The form of the rate equation for any reaction depends on the mechanism. But even in the simplest case, illustrated by Eq. (11-1), the Haldane relation illustrates the important fact that even though the kinetic parameters in a rate equation are all dependent on the properties of the enzyme, the apparent equilibrium constant for the catalyzed reaction is completely independent of the properties of the enzyme. Thus the parameter values (four in the case of Eq. (11-1), but more in more complicated cases) are not independent but must satisfy the constraint that the appropriate ratio must be consistent with the equilibrium constant [48,49].

Thus, K' for an enzyme-catalyzed reaction under a specified set of conditions can be calculated from the kinetic parameters. In fact, approximately 55 of the apparent equilibrium constants tabulated in the NIST reviews on the thermodynamics of enzyme-catalyzed reactions [38–45] were determined in this way. If the kinetic parameters are determined at several different pHs, $\Delta_r N_H$ can be calculated as a function of pH. If the kinetic parameters can be determined at two temperatures, $\Delta_r H'^\circ$ and $\Delta_r S'^\circ$ can also be calculated. This means that the form of the rate equation and the dependencies of kinetic parameters on pH and buffer composition have to be compatible with biochemical thermodynamics.

Transient methods can be used to determine rate constants of steps in the mechanism of an enzyme-catalyzed reaction. When this is achieved for all of the steps, the apparent equilibrium constants for

the steps can be calculated, and the apparent equilibrium constant for the catalyzed reaction is given by

$$K' = K'_1K'_2K'_3...K'_n \quad (11-3)$$

where $K'_1K'_2$ etc. are the apparent equilibrium constants for the first, second, etc. steps, and n is the number of steps in the mechanism.

The nomenclature for enzyme kinetics is described in an IUBMB Report [50].

12. Calorimetric measurements involving biochemical reactions

Calorimetry provides a direct method for measuring molar enthalpy changes for both chemical and biochemical reactions. It is important to recognize that there are essentially three parts to a thermochemical investigation [51]. For a reaction carried out at constant pressure, the first part involves the use of the calorimetric apparatus to measure the enthalpy change Δ_rH (units of J) for the reaction. The second part of the investigation is the chemical part in which one establishes that the actual reaction or process that occurs is the specified one and also in which one determines the extent of the reaction(s) that has occurred. The calorimetrically determined enthalpy change for the reaction $\Delta_rH(\text{cal})$ (units of J mol^{-1}) for the reaction is obtained as the ratio $\Delta H/n$, where n is the amount of substance that has reacted. Clearly, corrections must be made for any side reactions that may have occurred. Finally, there is often an adjustment made to bring the results to some standard set of conditions (temperature, pressure, ionic strength, etc.) or reference state, in order to obtain a standard enthalpy change Δ_rH° (units of J mol^{-1}) for a specified reaction.

Since biochemical reactions are generally carried out in a buffer, it is important to recognize that there may also be an enthalpy change associated with the reaction of protons or metal ions such as $Mg^{2+}(aq)$ with the buffer. Thus, the calorimetrically determined enthalpy change $\Delta_rH(\text{cal})$ is given by [52]

$$\Delta_rH(\text{cal}) = \Delta_rH'^\circ + \Delta_rN_H\Delta_rH^\circ(\text{H·Buffer}) + \Delta_rN_{Mg}\Delta_rH^\circ(\text{Mg·Buffer}). \quad (12-1)$$

The quantities $\Delta_rH^\circ(\text{buffer})$ and $\Delta_rH^\circ(\text{Mg·buffer})$ are, respectively, the standard enthalpy changes for the *appropriate* reaction involving the ionization of either $H^+(aq)$ or $Mg^{2+}(aq)$ from the complexed buffer:

$$\text{H·Buffer(aq)} = H^+(aq) + \text{Buffer}^-(aq) \quad (12-2)$$

$$\text{Mg·Buffer(aq)} = Mg^{2+}(aq) + \text{Buffer}^{2-}(aq) \quad (12-3)$$

Thus, the use of Eq. (12-1) to calculate $\Delta_rH'^\circ$ from the measured $\Delta_rH(\text{cal})$ requires a knowledge of Δ_rN_H. Also, if $Mg^{2+}(aq)$ reacts with the buffer, one also needs a value for Δ_rN_{Mg}. If one knows the pKs and metal-ion binding constants for all of the reactants in the biochemical reaction of interest, one can use an equilibrium model [25] to calculate K' as a function of pH. The quantity Δ_rN_H can then be calculated by means of Eq. (7-5). Alternatively, one can measure K' as a function of pH and then calculate Δ_rN_H. A third option is to perform calorimetric measurements with two different buffers, which differ substantially in their values of $\Delta_rH^\circ(\text{buffer})$. This gives two equations of the form (12-1) and allows one to calculate both $\Delta_rH'^\circ$ and Δ_rN_H. The latter two approaches are often necessary for reactions involving macromolecules where the pKs of the macromolecule are not known.

13. Recommendations for reporting experimental results

The usefulness and lasting value of an experimental investigation are made possible and enhanced by a careful reporting of the results of the investigation. In this regard, there are several matters that require attention:

- The identity of the principal substances used in the investigation must be stated. This can be accomplished by use of standard (e.g. IUPAC) and commonly accepted names, CAS Registry numbers, and by presenting the structures of the reactants and products. The last method is, by far, the most definitive method and avoids making the reader check the literature to obtain the structure(s). A combination of these methods is recommended. If substances have chiral centers, attention to which chiral forms are present is also required. If enzymes are used in a study they should be identified by EC numbers [12] and origin (e.g., species, tissue).
- The estimated purities of the materials and the methods of analysis used in the study should be reported.
- A description of the apparatus and procedures used in the investigation should be given.
- A clear reporting of the actual numerical values of the properties measured in the study is essential. While graphical presentation is very useful to illustrate a point, it is essential that the actual results be presented in numerical form. This saves the reader the difficulty and possible errors associated with having to extract numerical values from a graph.
- The reaction under investigation and its stoichiometry must be specified. For binding studies, one must state the basis for the assumed binding stoichiometry. If CO_2, N_2, NH_3, or other possible gaseous substances are involved in a reaction, it should be specified if their state in the reaction pertains to the gas phase or to the solution phase. If a reactant is bound to a surface or on a membrane, this should be clearly specified.
- A chemical reaction involves specific chemical (often ionic) species. It must balance both atoms and charges. A biochemical reaction involves sums of species and should not show charged species. Also, a biochemical reaction does not indicate that hydrogen atoms or magnesium atoms are conserved. However, C, N, O, and P, etc. are conserved. Thus, chemical and biochemical reactions have distinctly different physical and chemical bases. Therefore, they must not be confused. Nor should they be intermingled or combined. As stated in the 1994 Recommendations [3], one must be able to distinguish between these two types of reactions on sight.
- The equilibrium constant or apparent equilibrium constant must be clearly defined. This sometimes requires care in how one deals with water as a reactant. For reactions in aqueous media, the usual convention is to take the activity (or concentration) of water equal to unity. However, this is not the case for reactions carried out in non-aqueous media such as organic solvents. In this case, one must know the concentration of water in order to have a thermodynamically meaningful equilibrium constant as distinct from a ratio of concentrations for some of the reactants.
- When reporting the value of an equilibrium constant, particularly for an unsymmetrical reaction, one should specify the units of concentration used to calculate the equilibrium constant. It is recommended that the standard state either be 1 mol L^{-1} or 1 mol kg^{-1}. There must be no ambiguity in the direction of the reaction to which the equilibrium constant pertains.
- Concentration, units of mol L^{-1} is an accepted measure of the amount of a substance in a given volume of solution and molality (mol kg^{-1}) is appropriate for the amount in a given mass of solvent. Molality is little used in biochemistry, but it has the advantage that its value does not change with temperature and it is easily calculated from laboratory determinations of mass.
- It is essential that both the actual reaction and the conditions of measurement be specified. The conditions of measurement include, but are not limited to, the temperature, the pressure, pH, and the concentrations of the substances in solution. These quantities are measured directly. Other important quantities are pMg and ionic

strength. However, these are not measured directly and must be calculated. To perform these calculations, one needs information on the total concentrations of the reactants in the solution as well as the dissociation constants for all of the pertinent weak acids and magnesium complex ions. One also must make some assumption(s) about the activity coefficients used in these calculations. It is recommended that the experimental quantities, i.e. the temperature, the pressure, pH, and the concentrations of the substances in solution, be fully reported. If possible, one should also calculate the pMg and the ionic strength and report these values. A somewhat more extensive calculation can be performed that will lead to values of the equilibrium constant K and the standard molar enthalpy change $\Delta_r H°$ for a chemical reference reaction that corresponds to the (overall) biochemical reaction. If possible, it is also recommended that this calculation of K and $\Delta_r H°$ be performed. The choice of the chemical reference reaction is arbitrary. The method of calculation should be described and any auxiliary data used in these calculations should be included in the publication.

- For the study of biochemical reactions under "near physiological conditions," the following set of conditions have been widely used an exist as a de facto standard: $T = 310.15$ K, pH = 7.0, pMg = 3.0, and $I = 0.25$ mol L^{-1}. It is recognized that there is no unique set of physiological conditions and that for many purposes it will be necessary and desirable to study biochemical reactions under different sets of conditions.
- For calorimetric measurements, it is important to measure the extent of reaction.
- For equilibrium measurements, it is important to establish that the reaction under investigation has reached equilibrium. Product inhibition and a loss of enzyme activity are common phenomena that can lead to large systematic errors.
- The result of a measurement is also enhanced by a statement of its reliability or uncertainty. The uncertainty can be evaluated by the use of statistical methods and by a consideration of the possible systematic errors that might be associated with the measurement. Guidance on the estimation of uncertainties can be found in Ref. [53].
- IUPAC has published a "Guide to the Procedures for the Publication of Thermodynamic Data" [54] and CODATA has published a "Guide for the Presentation in the Primary Literature of Numerical Data Derived from the Experiments" [55]. Both of these "Guides" provide useful information on the reporting of physical property data. The importance of reporting essential information and results were emphasized in the 1972 IUPAC Recommendations [54]:

"The highly interdependent nature of thermodynamic data imposes special obligations upon the author of papers reporting the results of thermodynamic investigations. He must give enough information about his experiment to allow readers to appraise the precision and accuracy of his results so that they may be properly consolidated within the existing body of data in the literature. Further, as accepted values of physical constants change or as new thermodynamic data for related systems become available, subsequent investigators often can recalculate results if it is clear that they are based on good experiments for which adequate information is presented, however old they may be. For these reasons, an author's prime responsibility is to report his results in a form related as closely to experimentally observed quantities as is practical, with enough experimental details and auxiliary information to characterize the results adequately and to allow critical assessment of the accuracy claimed. For the convenience of the reader, the author may interpret and correlate the primary results as appropriate and present derived results in a form easy to utilize. However, such derived (or secondary) results *never* should be published at the cost of omitting the primary results on which they are based. Reference may be made to accessible earlier publications for some details."

14. List of symbols[1,2]

SI units are shown in parentheses. When a physical quantity does not have units, no units are given. Dimensions of matrices (row × column) are also indicated in parentheses.

$\boldsymbol{A}$	conservation matrix ($C \times N$)
$\boldsymbol{A}'$	apparent conservation matrix when the concentrations of one or more species are held constant ($C' \times N'$)
$\boldsymbol{A}''$	apparent conservation matrix when the concentrations of one or more species and one or more reactants are held constant ($C'' \times N''$)
B	empirical constant in the extended Debye–Hückel equation (1.6 $kg^{1/2}$ $mol^{-1/2}$)
$[B_i]$	concentration of reactant i (SI base units are mol m^{-3}; commonly used units are mol L^{-1} or mol dm^{-3})
$[B_j]$	concentration of species j (SI base units are mol m^{-3}; commonly used units are mol L^{-1} or mol dm^{-3})
$c°$	standard concentration (SI base units are 1 mol m^{-3}; commonly used units are 1 mol L^{-1} or 1 mol dm^{-3})
C	number of components in a reaction system
C'	apparent number of components in a reaction system when the concentrations of one or more species are held constant
C''	apparent number of components when the concentrations of one or more species and one or more reactants are held constant
E	electrode potential of a cell (formerly called electromotive force or electric potential difference) or reduction potential (V)
$E°$	standard electrode potential of a cell (formerly called standard electromotive force or standard electric potential difference) or standard reduction potential (V)
E'	apparent electrode potential of a cell or apparent reduction potential at a specified pH (V)
$E'°$	standard apparent electrode potential of a cell or standard apparent reduction potential at a specified pH (V)
F	Faraday constant (96 485.339 9 C mol^{-1})
G	Gibbs energy of a system at specified T, P, and ionic strength (J)
G'	transformed Gibbs energy of a system at specified T, P, ionic strength, and concentrations of one or more species (J)
G''	further transformed Gibbs energy of a system at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants (J)
$\Delta_f G_j°$	standard Gibbs energy of formation of species j at specified T, P, and ionic strength (J mol^{-1})
$\Delta_f G_j$	Gibbs energy of formation of species j at specified T, P, and ionic strength (J mol^{-1})
$\Delta_r G$	reaction Gibbs energy of chemical reaction (J mol^{-1})
$\Delta_r G°$	standard reaction Gibbs energy of chemical reaction (J mol^{-1})
$\Delta_f G_i'°$	standard transformed Gibbs energy of formation of reactant i at specified T, P, ionic strength, and specified concentrations of one or more species (J mol^{-1})

[1] A subscript "m" is often used in the literature of physical chemistry to denote molar quantities. Since molar quantities are clearly identified by their units in this table and by the context of the equations, this subscript has not been used in this document. However, to avoid ambiguity or if the units are not specified, we recommend the use of a subscript "m" to denote molar quantities.

[2] The values of equilibrium constants for reactions that are not symmetrical depend on the units and standard state on which an equilibrium constant is based. A way of denoting this is to use K_c rather than K. If the equilibrium constant is based on molality or mole fraction, one can use K_m or K_x, respectively. Similarly, the values of the ionic strength I, pK, pH, pMg, and the activity coefficient γ also depend on whether one has used a concentration, molality, or mole fraction basis. Thus, when necessary, these quantities can also be distinguished by the addition of a subscript "c", "m", or "x." In this document, the quantities K, pK, I, pH, pMg, and γ are all based on concentration and the subscript "c" has not been used.

$\Delta_f G_j'^\circ$ standard transformed Gibbs energy of formation of species j at specified T, P, ionic strength, and specified concentrations of one or more species (J mol^{-1})

$\Delta_f G_i''^\circ$ standard further transformed Gibbs energy of formation of a pseudoisomer group of reactants at specified T, P, ionic strength, and specified concentrations of one or more species and one or more reactants (J mol^{-1})

$\Delta_r G'$ transformed Gibbs energy of a biochemical reaction (J mol^{-1})

$\Delta_r G'^\circ$ standard transformed Gibbs energy of reaction at a specified temperature, pH, ionic strength (J mol^{-1})

$\Delta_r G''$ further transformed Gibbs energy of reaction at specified concentrations of one or more reactants (J mol^{-1})

$\Delta_r G''^\circ$ standard further transformed Gibbs energy of reaction at specified concentrations of one or more reactants (J mol^{-1})

H enthalpy of a system at specified T, P, and ionic strength (J)

H' transformed enthalpy of a system at specified T, P, ionic strength, and concentrations of one or more species (J)

H'' further transformed enthalpy of a system at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants (J)

$\Delta_r H$(cal) calorimetrically determined enthalpy of reaction that includes the enthalpies of reaction of H^+ and Mg^{2+} (consumed or produced) with any buffer in solution (J mol^{-1})

$\Delta_r H^\circ$ standard enthalpy of reaction (J mol^{-1})

$\Delta_f H_j^\circ$ standard enthalpy of formation of species j (J mol^{-1})

$\Delta_r H'^\circ$ standard transformed enthalpy of reaction at a specified concentration of a species (J mol^{-1})

$\Delta_r H'$ transformed enthalpy of reaction at a specified concentration of a species (J mol^{-1})

$\Delta_f H_i'^\circ$ standard transformed enthalpy of formation of reactant i at a specified concentration of a species (J mol^{-1})

I ionic strength (mol L^{-1} or mol dm^{-3})

K equilibrium constant written in terms of concentrations of species at specified T, P, and ionic strength

K' apparent equilibrium constant written in terms of concentrations of reactants (sums of species) at specified T, P, ionic strength and concentrations of one or more species

K'' apparent equilibrium constant written in terms of concentrations of pseudoisomer groups (sums of reactants) at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants

K_A Michaelis constant for A in an enzyme-catalyzed reaction (SI base units are mol m^{-3}; commonly used units are mol L^{-1} or mol dm^{-3})

K_a acid dissociation constant

K_{ref} equilibrium constant for a chemical reference reaction

n total amount of species in a system (mol)

n_j amount of species j (mol)

n_i' amount of reactant i (sum of species) (mol)

n_i'' amount of pseudoisomer group i (sum of reactants) (mol)

n_c amount of a component C in a system (mol)

n_c' amount of an apparent component C at specified concentrations of one or more species (mol)

n_c'' amount of an apparent component C at specified concentrations of one or more species and one or more reactants (mol)

n'(H) amount of hydrogen atoms in a system, not counting H^+ (mol)

N number of different kinds of species in a system

N' number of different reactants (sums of species) in a system

N'' number of different pseudoisomer groups of reactants in a system

N_{iso} number of isomers in an isomer group or pseudoisomers in a pseudoisomer group

$N_H(j)$ number of hydrogen atoms in species j

$N_{ATP}(i)$ number of ATPs in reactant i (can be positive or negative)

$N_{Mg}(j)$ number of magnesium atoms in a molecule of j

$\overline{N}_H(i)$ average number of hydrogen atoms bound by a molecule of i

$\overline{N}_{Mg}(i)$ average number of magnesium atoms bound by a molecule of i

$\overline{N}_{ATP}(i)$ rate of change of n_c(ATP) with respect to the amount of pseudoisomer group i

$\Delta_r N_H$ change in binding of H^+(aq) in a biochemical reaction at specified T, P, ionic strength and concentrations of one or more species

$\Delta_r N_{Mg}$ change in the binding of Mg^{2+}(aq) in a biochemical reaction at specified T, P, pH, and ionic strength

P pressure (bar)

P binding polynomial (partition function)

P_j partial pressure of species j (bar)

pH $-\log_{10}[H^+]$ at specified T, P, and ionic strength

pK $-\log_{10}K$ for the dissociation of an acid at specified T, P, and ionic strength

pMg $-\log_{10}[Mg^{2+}]$ at specified T, P, and ionic strength

q heat flow into a system (J)

Q reaction quotient at T and P

Q' apparent reaction quotient at specified T, P, pH, pMg, and ionic strength

r_i mole fraction of isomer i within an isomer group or pseudoisomer i within a pseudoisomer group

R gas constant (8.314 472 J K^{-1} mol^{-1})

R number of independent reactions in a system described in terms of species

R' number of independent reactions in a system described in terms of reactants (sums of species)

R'' number of independent reactions in a system described in terms of pseudoisomer groups of reactants

S entropy of a system at specified T, P, and ionic strength (J K^{-1})

S' transformed entropy of a system at specified T, P, ionic strength, and concentrations of one or more species (J K^{-1})

S'' further transformed entropy of a system at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants (J K^{-1})

ΔS change in entropy in a change of state of a system (J K^{-1} mol^{-1})

$S(j)$ entropy of species j (J K^{-1} mol^{-1})

$S'(i)$ transformed entropy of reactant i (J K^{-1} mol^{-1})

$S^\circ(j)$ standard entropy of species j (J K^{-1} mol^{-1})

$S'^\circ(i)$ standard transformed entropy of reactant i (J K^{-1} mol^{-1})

$\Delta_r S$ reaction entropy of a chemical reaction (J K^{-1} mol^{-1})

$\Delta_r S^\circ$ standard reaction entropy of a chemical reaction (J K^{-1} mol^{-1})

$\Delta_f S_j^\circ$ standard entropy of formation of species j (J K^{-1} mol^{-1})

$\Delta_r S'$ transformed reaction entropy of a biochemical reaction (J K^{-1} mol^{-1})

$\Delta_r S'^\circ$ standard transformed reaction entropy (J K^{-1} mol^{-1})

$\Delta_f S'^\circ(j)$ standard transformed entropy of formation of species j (J K^{-1} mol^{-1})

T temperature (K)

U internal energy (J)

U' transformed internal energy (J)

v velocity (rate) of an enzyme-catalyzed reaction

V volume (m^3, L, or dm^3)

V_f limiting velocity (rate) of the forward reaction for an enzyme-catalyzed reaction

V_r limiting velocity (rate) of the reverse reaction for an enzyme-catalyzed reaction

w work done on a system (J)

z_j charge number of ion j

α Debye–Hückel constant (1.17 582 kg$^{1/2}$ mol$^{-1/2}$ at 298.15 K)

γ_j activity coefficient of species j

μ_j chemical potential of species j at specified T, P, and ionic strength (J mol^{-1})

μ_i' transformed chemical potential of reactant i at specified T, P,

ionic strength, and concentrations of one or more species (J mol^{-1})

μ_i'' further transformed chemical potential of pseudoisomer group i at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants (J mol^{-1})

μ_j° standard chemical potential of species j at specified T, P, and ionic strength (J mol^{-1})

$\mu_i'^\circ$ standard transformed chemical potential of reactant i (J mol^{-1})

$\mu_i''^\circ$ standard further transformed chemical potential of pseudoisomer group i at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants (J mol^{-1})

μ_i chemical potential of component i at specified T, P, and ionic strength (J mol^{-1})

μ_i' transformed chemical potential of component i at specified T, P, ionic strength, and concentrations of one or more species (J mol^{-1})

μ_i'' further transformed chemical potential of component i at specified T, P, ionic strength, and concentrations of one or more species and one or more reactants (J mol^{-1})

ν_j stoichiometric number of species j in a chemical reaction

ν_i' stoichiometric number of reactant i in a biochemical reaction at specified pH

ν_i'' apparent stoichiometric number of reactant i (sum of species) when the concentration of a reactant has been specified

$|\nu_e|$ number of electrons in a half reaction

ξ extent of chemical reaction (mol)

ξ' extent of biochemical reaction (mol)

References

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