

## RECOMMENDATIONS FOR MEASUREMENT AND PRESENTATION OF BIOCHEMICAL EQUILIBRIUM DATA\*

PREPARED BY THE INTERUNION COMMISSION ON BIOTHERMODYNAMICS\*\*

Equilibrium data are of importance in describing and understanding biochemical systems. At present there is marked variation among different investigators in the choice of experimental conditions for the study of similar or identical reactions and in the manner of reporting the data. In many cases, the description of the system does not provide all the essential information that would permit reproduction of the experiments. This can lead to confusion and difficulties in correlating the results of different workers.

Equilibrium studies in biochemistry involve special problems that are not encountered in general chemistry. The attainment of biochemical equilibria commonly involves the addition of a specific enzyme to the system to catalyze the reaction studied; sometimes two or more enzymes must be added. In addition, the enzyme may require the presence of certain cofactors, such as metal ions. The reactants, or products, or both, may bind or release protons or other ions during the reaction under study. Thus, the experimental system must be described with particular care.

The acquisition and reporting of meaningful thermodynamic data relating to biochemical systems, as well as to other complex reacting systems, involves two fundamental problems.

1. It is not always possible to define the reacting species precisely.
2. Even in cases where the reacting species can be defined, their thermodynamic activities frequently cannot be determined.

In this report, we offer several recommendations with the aim of increasing the usefulness of biochemical equilibrium data and coordinating the results of different investigators. These recommendations include a set of standard conditions which would facilitate the attainment of a common body of knowledge of a wide range of biochemical equilibria. This does not preclude the choice of special experimental

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conditions that may be appropriate for certain reactions; but whenever possible these reactions should also be studied under the recommended standard conditions. To avoid confusion in interpretation we also recommend standardization of terminology, symbols, and units in the presentation of such data.

For other discussions of the presentation of numerical data and of thermodynamic data derived from experiments we call the attention of the reader to guides prepared by CODATA [1] and IUPAC [2].

## PART I. STANDARD CONDITIONS FOR EQUILIBRIUM MEASUREMENTS

True thermodynamic equilibrium constants are defined in terms of activities of the reactants and products. In many systems of biochemical interest it is not possible to evaluate the activities of all components. It is therefore frequently necessary to calculate equilibrium constants in terms of concentrations. The proper quotient of equilibrium concentrations is acceptably constant for many purposes, and will be referred to in this document as the concentration equilibrium constant, with the symbol  $K_c$ . However, it should be recognized that values for such equilibrium constants,  $K_c$ , and corresponding Gibbs energy changes,  $\Delta G_c^0$ , may not be truly constant as the composition of the system is changed\*. It is essential that reported values for such quantities be accompanied by a complete and accurate description of the composition of the reacting system, and the methods by which the composition was established. References to prior work will in many cases provide adequate descriptions of these methods.

### *Experimental conditions*

It is recommended that measurements be made at 25 °C (or 37 °C) and an ionic strength brought to  $0.10 \text{ mol} \cdot \text{dm}^{-3}$  with potassium chloride\*\*, using the lowest effective buffer concentration. If these conditions are not practicable, conditions should be chosen that are well defined and can be maintained constant throughout a series of experiments. Reagents should be as pure as practicable, and the method of purification, estimated purity, and source of each reagent should be given. The existence of a true equilibrium condition should be demonstrated by approaching the equilibrium state from both directions and by making determinations at several different concentrations of the reactants. It is desirable also to study the effect of variation of the ionic strength and the presence of specific salt effects. Complete or representative experimental data from which equilibrium constants are derived should be reported.

**Temperature.** A standard temperature of 25 °C (298.15 K) is preferred to permit comparison with most available chemical data. The numerical values of the equilibrium constant at both 25 °C and 37 °C should be reported when possible.

It is desirable to measure equilibrium constants at several temperatures. The

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\* The standard Gibbs energy change,  $\Delta G^0$ , is now the correct term for what is frequently called the standard Gibbs free energy change or simply the free energy change and often given the symbol  $\Delta F^0$ .

\*\* The recommended unit of concentration is  $\text{mol} \cdot \text{dm}^{-3}$ , commonly denoted by M in the biochemical literature.

standard enthalpy and entropy changes for a specific reaction can then be calculated from the temperature dependence of the equilibrium constant. However, when such experiments are performed it is essential to take account of changes in the dissociation constants of reactants and buffers with changing temperature and the resulting changes in pH and in the concentrations of dissociating species. It may be noted that calorimetry is generally a more accurate technique for determination of the enthalpy change of a reaction. The calorimetrically determined enthalpy change must be corrected for the enthalpy change from any reaction of a buffer involving protons that are taken up or given off in the reaction.

*Buffer and pH.* If only a limited number of measurements are to be made, they should be carried out at  $\text{pH} = 7.0$  and, if possible, also at a pH value at which the apparent equilibrium constant  $K'_c$ , has little or no dependence on pH. ( $K'_c$  is defined in a later section.) If direct measurements at  $\text{pH} = 7.0$  are not practicable, the calculated values for this pH should be reported. The procedure used in making these calculations must be carefully described. Care should be taken that the solution is adequately buffered so that the pH is well defined throughout the experiment. It is desirable to determine the effect of varying the nature and concentration of the buffer in order to identify buffer effects. Buffers that are known to interact with reactants (including macromolecules) or salts, such as phosphate or pyrophosphate in the presence of divalent metal ions, should be avoided.

*Complexations with ions.* The equilibrium constants for complexation of ions with reactants, such as magnesium ion with phosphate compounds, should be determined under the conditions of the experiments and the concentrations of reactants should be corrected for complexation. If this is not practicable, there are three alternatives: (a) Metal ions that are necessary for activation of enzymes may be added in trace amounts that are sufficient to permit attainment of equilibrium but are too small to change the concentrations of reactants significantly by complexation. (b) Measurements may be carried out at a concentration of metal ions such that essentially all of one (or more) of the reactants exists in the complexed form and the degree of complexation of other reactants is not significant; i.e. the equilibrium constant and standard Gibbs energy change are determined for the reaction involving the complexed species. (c) The measurements may be carried out under physiological conditions, to obtain equilibria and standard Gibbs energy changes that are directly applicable to biological systems.

In any case, the concentrations of metal ions should be specified and, when possible, equilibrium constants should be reported corresponding to the three situations above, i.e. uncomplexed reactants, fully complexed reactants (e.g.  $\text{Mg} \cdot \text{ATP}$ ), and physiological conditions. In view of the limited availability and occasional unreliability of equilibrium constants for ion complexation, it is highly desirable to determine directly the effect of variation of the concentration of metal ions under the experimental conditions employed. In some cases it may be necessary to correct for complexation of reactants by appropriate extrapolation procedures or to use special experimental conditions to avoid complexation of the reactants with components of the reaction medium. For example, tetramethylammonium chloride may be used instead of potassium chloride for measurements with polyphosphates or other poly-anions that complex with alkali ions.

*General considerations in reporting equilibrium data*

Consider the following reaction



where  $a$ ,  $b$ ,  $c$ , and  $d$ , respectively, are the stoichiometric coefficients of components A, B, C, and D. In this case the concentration equilibrium constant is

$$K_c = \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b} \quad (2)$$

and the corresponding standard Gibbs energy\* change is

$$\Delta G_c^0 = -RT \ln K_c \quad (3)$$

where  $[A]$ ,  $[B]$ , etc. are the concentrations of distinct molecular components involved in the chemical reaction,  $R$  is the gas constant and  $T$  is the thermodynamic temperature.

Water as a reactant in *dilute aqueous* solutions provides a special problem. In order to conform to the general principles of dilute solutions, the value for the factor  $[H_2O]$  in equilibrium expressions should be taken as unity in such cases (see the last paragraph of Part I).

In some cases it is known that the designated concentration of a component includes several distinct molecular species (e.g.  $[C]$  is  $[CH_3COOH] + [CH_3COO^-]$ ) but insufficient information exists to allow a further description of the equilibrium system. For example, in a buffered system it may not be possible to evaluate the participation of the  $H^+$  ion in all of the possible equilibria. However, if the  $[H^+]$  may be maintained constant it is possible to write an apparent equilibrium constant,  $K'_c$ , in which the  $[H^+]$  does not appear explicitly. Such an apparent equilibrium constant will be pH-dependent and will generally have a different value from  $K_c$  (Equation 2) which will be pH-independent. The  $K'_c$  is written in terms of the total concentrations of the measurable components

$$K'_c (\text{pH} = x, \text{etc.}) = \frac{[C_1 + C_2 + \dots]^c [D_1 + D_2 + \dots]^d}{[A_1 + A_2 + \dots]^a [B_1 + B_2 + \dots]^b} \quad (4)$$

and an apparent standard Gibbs energy change

$$\Delta G_c^{0'} (\text{pH} = x, \text{etc.}) = -RT \ln K'_c \quad (5)$$

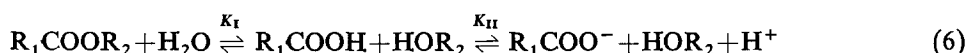
The value of  $K'_c$  is subject to certain constraints. The value of pH, and perhaps that of

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\* Equation 3 implies that the equilibrium in question is for a "dilute-ideal" solution, in which the activity coefficients of all reactants and products are independent of concentration over the concentration range under consideration. Although this condition cannot be expected to hold rigorously in any actual system, in practice  $\Delta G_c^0$  is frequently found to be constant within the experimental error of the measurements in biochemical systems. Therefore, we shall refer to  $\Delta G_c^0$  as a "standard Gibbs energy change," although it should be recognized that this usage is an approximation. It is also to be noted that the argument of a logarithm (e.g.,  $K_c$  in Equation 3) must be dimensionless. For concentration equilibrium constants this can be achieved by using "relative concentrations," that is, concentration divided by a standard concentration = 1. This operation is of importance in principle, but can be ignored in practice. It is necessary, however, that the standard state concentration units be explicitly described when reporting such values.

the concentration of certain metal ions, or other factors must be fixed and specified in order to obtain a definite  $K'_c$  value. The values of these constraints must be stated, as indicated above, but these constraining factors do not explicitly appear among the concentrations of components on the right hand side of Equation 4. Usually in biochemistry subscript  $c$  in  $K_c$  can be deleted. However, it is recommended that the notation "apparent" should never be left out in connection with  $K'_c$  and  $\Delta G'_c$ .

A simple example should help clarify the situation. Consider the reaction:



The concentration equilibrium constants and standard Gibbs energy changes for steps I and II are

$$K_I = \frac{[R_1COOH][HOR_2]}{[R_1COOR_2][H_2O]} \quad (7)$$

Since the factor for water in Equation 7 is taken as unity in *dilute aqueous solutions*, it may be omitted in writing the equations for equilibria in such systems.

$$\Delta G_I^0 = -RT \ln K_I \quad (8)$$

$$K_{II} = \frac{[R_1COO^-][HOR_2][H^+]}{[R_1COOH][HOR_2]} = \frac{[R_1COO^-][H^+]}{[R_1COOH]} \quad (9)$$

$$\Delta G_{II}^0 = -RT \ln K_{II} \quad (10)$$

The concentration equilibrium constant and standard Gibbs energy change for the overall reaction are

$$K = \frac{[R_1COO^-][HOR_2][H^+]}{[R_1COOR_2]} = K_I K_{II} \quad (11)$$

$$\Delta G^0 = -RT \ln K \quad (12)$$

However, an apparent equilibrium constant at some  $pH = x$  can be written in which the concentrations of  $R_1COO^-$  and  $R_1COOH$  are summed and do not have to be differentiated.

$$K' (pH = x) = \frac{([R_1COO^-] + [R_1COOH])[HOR_2]}{[R_1COOR_2]} \quad (13)$$

The corresponding standard Gibbs energy change is

$$\Delta G'^0 (pH = x) = -RT \ln K' \quad (14)$$

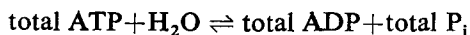
In cases where the details of the chemical equilibria are known the interconversion between  $K$  and  $K'$  is generally straightforward. For the example just described

$$[R_1COOH] = \frac{[R_1COO^-][H^+]}{K_{II}} \quad (15)$$

and

$$K' (pH = x) = \frac{[R_1COO^-](1 + [H^+]/K_{II})[HOR_2]}{[R_1COOR_2]} = K \left(1 + \frac{[H^+]}{K_{II}}\right) \cdot \frac{1}{[H^+]} \quad (16)$$

The well known problem of the standard Gibbs energy change of ATP hydrolysis to ADP and inorganic phosphate ( $P_i$ ) furnishes a more complex example of concentration and apparent equilibrium constants. The overall reaction may be written



Thus, the apparent equilibrium constant and  $\Delta G^{0'}$  values at some specified pH are:

$$K' = \frac{[\text{total ADP}] [\text{total } P_i]}{[\text{total ATP}]} \quad (17)$$

$$\Delta G^{0'} = -RT \ln \frac{[\text{total ADP}] [\text{total } P_i]}{[\text{total ATP}]} \quad (18)$$

Data are available to convert these apparent constants into concentration constants. In order to deal with these phosphate compounds in a medium related to their biological environment, it is necessary to consider their interactions with protons and with magnesium ions. At pH values near 7 and at physiological  $[\text{Mg}^{2+}]$  values, it is found that each of the three phosphates can be described by one acidic dissociation, and each acid and its conjugate base may bind 1  $\text{Mg}^{2+}$  ion. Thus the total concentrations in  $K'$  and  $\Delta G^{0'}$  may be resolved as follows:

$$[\text{total ATP}] = [\text{ATP}^{4-}] + [\text{ATP}^{3-}] + [\text{ATPMg}^{2-}] + [\text{ATPMg}^-] \quad (19)$$

$$[\text{total ADP}] = [\text{ADP}^{3-}] + [\text{ADP}^{2-}] + [\text{ADPMg}^-] + [\text{ADPMg}] \quad (20)$$

$$[\text{total } P_i] = [P_i^{2-}] + [P_i^-] + [P_i\text{Mg}] + [P_i\text{Mg}^+] \quad (21)$$

In fact  $[P_i\text{Mg}^+]$  is always so small as to be negligible, and  $[P_i\text{Mg}]$  is of minor importance.

The three  $pK_a$  values involved—one for each of the compounds ATP, ADP, and  $P_i$ —are known, as are the association constants of the various species for  $\text{Mg}^{2+}$  ion. We can then formulate the reaction in terms of the relations between particular species of reactants and products. For instance in the limiting case of high pH ( $> 8$ ) and  $[\text{Mg}^{2+}] = 0$ , the predominant reaction becomes:



It would be equally valid to formulate the reaction in terms of the equation:



and other choices are obviously possible, provided that the equations balance with respect to total charge and stoichiometry. We note that the equilibrium constants of all such reactions must be independent of pH, whether or not  $[\text{H}^+]$  enters explicitly into the reaction. Formulation of such equilibrium constants implies nothing as to the mechanism of the reaction in this or any other case.

If we choose to formulate the reaction in terms of Equation 22, we have:

$$K_{\text{ATP}^{4-}} = \frac{[\text{ADP}^{3-}] [P_i^{2-}] [\text{H}^+]}{[\text{ATP}^{4-}]} \quad (24)$$

In the limiting case where  $[\text{Mg}^{2+}] = 0$ , we can then write for the relation between  $K'$  (of Equation 17) and  $K_{\text{ATP}^{4-}}$  (Equation 24):

$$\begin{aligned}
 K' (\text{pH} = x, [\text{Mg}^{2+}] = 0) &= \frac{([\text{ADP}^{3-}] + [\text{ADP}^{2-}]) ([\text{P}_i^{2-}] + [\text{P}_i^-])}{([\text{ATP}^{4-}] + [\text{ATP}^{3-}])} \\
 &= K_{\text{ATP}^{4-}} \frac{\left(1 + \frac{[\text{H}^+]}{K_\beta}\right) \left(1 + \frac{[\text{H}^+]}{K_\gamma}\right)}{\left(1 + \frac{[\text{H}^+]}{K_\alpha}\right) [\text{H}^+]} \quad (25)
 \end{aligned}$$

where  $K_\alpha$ ,  $K_\beta$ , and  $K_\gamma$  are, respectively, the acidic dissociation constants of  $\text{ATP}^{3-}$ ,  $\text{ADP}^{2-}$ , and  $\text{P}_i^-$ .

Since all the constants in Equation 25 are known, the apparent equilibrium constant Equation 17 is thus explicitly related to the pH-independent constant  $K_{\text{ATP}^{4-}}$ . In the presence of  $\text{Mg}^{2+}$  ion the analysis is in principle exactly similar but is much more complicated in detail. A detailed discussion is given for instance by Alberty [3].

We note that the usefulness of Equation 17 depends upon the fact that it is possible to determine analytically the *total* concentrations of ATP, ADP, and  $\text{P}_i$ , without distinguishing between the different forms listed in Equations 19, 20, and 21.

We recommend that concentration equilibrium constants and corresponding standard Gibbs energy changes be reported when the reaction can be completely and accurately defined. In cases where this is impossible corresponding apparent quantities can be reported, if possible over a range of pH values and other constraining variables. In any case the values must be accompanied by a precise definition of the reaction scheme assumed.

**Quantities.** It is recommended that values of equilibrium constants and standard Gibbs energy changes should normally be reported based on concentrations (amount of substance divided by volume, with units of moles per cubic decimetre) except for the proton factor, for water in aqueous systems in which water is involved as a reactant or product, for gases, or for solids. For special purposes it may be desirable to report additional values that are based on molalities, mole fractions, or partial pressures; in any case the unit employed should be clearly identified. The use of equilibrium constants based on concentrations expressed in such units as millimoles or micromoles per cubic decimetre (litre) should be avoided.

It is recommended that for reactions in dilute aqueous solutions the factor for  $\text{H}_2\text{O}$  be taken as unity, in the absence of a measurement of its relative activity. An actual concentration such as the conventional  $55.5 \text{ mol} \cdot \text{dm}^{-3}$  should only be used for liquid water for well defined reasons. It is recommended that  $10^{-\text{pH}}$  be used for the proton factor. The conventions chosen for the liquid water and the proton factor must always be clearly and explicitly stated. For gases the partial pressure in atmospheres or (if the partial pressure is expressed in kilopascals) the ratio of the partial pressure to 101.325 kPa should be used. Gibbs energies should be reported in joules per mole ( $\text{J} \cdot \text{mol}^{-1}$ ). Until this unit comes into general use, results may in addition be reported in thermochemical calories per mole ( $1 \text{ cal}_{\text{th}} = 4.184 \text{ J}$ ).

## PART II. SYMBOLS, UNITS, AND TERMINOLOGY

### *General*

The precision of statement, the universal intelligibility, and the enduring

quality of a thermodynamic document will be enhanced if the symbols, units, and terminology used in it are those recommended by an international standardizing body. The following information summarizes recommendations of international scientific bodies in the physical and measurement sciences, selected for particular relevance to the thermodynamics of biological processes. The author is referred to recommendations of the IUPAC [4]\* for more detailed lists of recommended symbols and terminology for physicochemical quantities, and for a discussion of the International System of Units (SI). The SI is also recommended by the International Organization for Standardization (ISO), which provides rules for their use [5]. Other descriptions of the SI are provided by the International Bureau of Weights and Measures [6].

In adhering to these recommendations for the presentation of scientific data, authors are urged:

1. To use the recommended name of each physical quantity and the preferred symbol, as far as possible.
2. To use SI units and their internationally accepted symbols [4–6].
3. To use functional expressions where feasible, rather than subscripts or superscripts, for specifying substance, physical state, and temperature to which the quantity refers, e.g. for a process such as Reaction 1

$\Delta G_1^{0'}$  (pH = 7.0,  $I_c = 0.10 \text{ mol} \cdot \text{dm}^{-3}$ , at 25 °C)

or for a dissolved substance

$C_p(\text{C}_2\text{H}_5\text{OH}, \text{in } \text{H}_2\text{O}, 25 \text{ }^\circ\text{C})$

4. To indicate both numerical values and units in referring to physical quantities in the text, and in labels for axes of graphs and rows and columns of tables.

The preferred convention for statements in the text is:

physical quantity = numerical value  $\times$  unit

e.g.

$\Delta G^{0'}(\text{pH} = 7.0, I_c = 0.10 \text{ mol} \cdot \text{dm}^{-3}, \text{at } 25 \text{ }^\circ\text{C}) = -8.7 \text{ kJ} \cdot \text{mol}^{-1}$ .

The preferred label for tables and graphs, obtained by rearrangement of the equation above is:

physical quantity/unit = numerical value

that is, in the equation  $\Delta G/\text{kJ} \cdot \text{mol}^{-1} = -8.7$ , the left hand side is the label and the

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\* Ref. 4 gives a summary of the International System of Units (SI) and recommendations for the usage of these units including reference to usage of other commonly found non-SI units. It also lists recommended names and symbols for quantities relating to space and time, mechanical properties, molecular masses and concentrations, thermodynamics, electricity and magnetism, electrochemistry, light and electromagnetic radiation, and transport properties.

Recommendations are also given in Ref. 4 for subscripts and superscripts and for format of presenting formulas, symbols, units, numerical values, mathematical symbols, and relationships. Special chapters discuss notation and conventions for electromotive force and electrode processes, pH measurements, and reaction rates and related quantities. Also of interest to the biochemist and biothermodynamicist is an appendix which gives recommendations concerning the definitions of activities and related properties in aqueous solutions as well as other phases.



right hand side is the numerical quantity to be tabulated. If a power of 10 is used in addition to or in place of a prefix on the units the power of 10 should be shown correctly with the units (thus  $\Delta G/\text{J} \cdot \text{mol}^{-1} = -0.087 \times 10^5$ , or  $\Delta G/10^5 \text{ J} \cdot \text{mol}^{-1} = -0.087$ , or any other algebraically correct form).

Values other than SI units may be included in parentheses when it is felt this will improve communication between the author and his readers. This may be considered desirable when there is still widespread current usage of a non-SI unit for a certain quantity; such as the calorie or the atmosphere. When a non-SI unit is used, the author should define it in terms of SI units.

The symbols for physical quantities should be printed in italic (sloping) type or underlined in typescript and the symbols for units should be printed in roman (upright) type.

The usage of these recommendations is illustrated below for certain specific applications in thermodynamic measurements.

No attempt will be made here to discuss all of the names and symbols for physical quantities, which are adequately described in the references given. Our attention will be directed toward those for which the International recommendations form a distinct variation from much established practice.

### *Temperature*

Thermodynamic temperatures and temperature differences are expressed in kelvins, symbol K, not degrees Kelvin or °K. Celsius temperatures and temperature differences are expressed in degrees Celsius, symbol °C. The degree Celsius, which is identical to the kelvin, is sometimes improperly called the degree “centigrade.”

### *Pressure*

The unit of pressure is the pascal, which is one newton per square metre ( $1 \text{ Pa} = 1 \text{ N} \cdot \text{m}^{-2}$ ). A convenient unit for many pressure measurements is the kilopascal (symbol kPa). The biological thermodynamicist should recognize that the common thermodynamic term,  $pV$ , is an energy term, and if  $p$  is in pascals and  $V$  is in cubic metres, then the  $p \cdot V$  product is obtained directly in joules. No combination of customary non-SI units avoids the use of a conversion factor; and such awkward energy units as litre-atmosphere are avoided by the recommended usage.

The commonly found units mmHg or Torr, and their submultiples, should be avoided.

The standard atmosphere is defined as 101 325 pascals ( $1 \text{ atm} = 101.325 \text{ kPa}$ ). This is a non-SI unit. In calculating equilibrium constants and standard thermodynamic functions based on pressure measurements it should be recognized that the accepted standard pressure is 101.325 kPa, which is often called one atmosphere in this context.

### *Energy*

Energy measurements, including all thermal measurements, should be reported in joules (J), kilojoules (kJ), or millijoules (mJ) as appropriate. In cases where energies are also expressed in thermochemical calories ( $\text{cal}_{\text{th}}$ ) or a multiple or sub-multiple, the author should state the conversion factor used ( $1 \text{ cal}_{\text{th}} = 4.184 \text{ J}$ ). The “nutritional” or “large” calorie (sometimes abbreviated “Cal” and equal to one

kilocalorie) has little use outside of its special area, and its use should be avoided. Other definitions of calorie have little or no use in biochemistry or in other thermochemical work.

The author should realize that the use of calories is on the decline because of the acceptance of the SI units, so that data reported in calories will probably require conversion relatively soon.

For other units of measure, the author is referred to IUPAC and BIPM recommendations [4–6].

### *Composition of solutions*

For thermodynamic applications the composition of a solution is commonly described in terms of concentration, molality, or mole fraction. Other descriptors, such as mass fraction or volume fraction, may be found convenient for special applications. All of these should be clearly distinguished, and the solutions used should be unambiguously described using proper units.

The concentration of a solute substance B is the amount of B (moles) divided by the volume of the solution. Accepted symbols are  $c_B$  and  $[B]$ . A convenient unit of concentration is  $\text{mol} \cdot \text{dm}^{-3}$ . Concentration is sometimes called “molarity.” A solution with a concentration of  $0.1 \text{ mol} \cdot \text{dm}^{-3}$  is often called a 0.1 molar solution or a 0.1 M solution. Because the term molarity and the unit M are liable to be confused with molality, the term concentration and the unit  $\text{mol} \cdot \text{dm}^{-3}$  are preferred.

The mass concentration of a solute substance B is the mass of B divided by the volume of the solution. An accepted symbol is  $\rho_B$  and an appropriate unit is  $\text{kg} \cdot \text{m}^{-3}$ .

The molality of solute substance B is the amount of B (moles) divided by the mass of solvent. An accepted symbol is  $m_B$  and an appropriate unit is  $\text{mol} \cdot \text{kg}^{-1}$ . A solution having a molality equal to  $0.1 \text{ mol} \cdot \text{kg}^{-1}$  is sometimes called a 0.1 molal solution or a 0.1 m solution. Because of possible confusion of “molal” with “molar” and because m is the symbol for the metre, the unit  $\text{mol} \cdot \text{kg}^{-1}$  is preferred.

The mole fraction of substance B is the amount of B (moles) in a solution divided by the total amount (moles) of all substances in the solution. Accepted symbols are  $x_B$  and  $y_B$ , where  $x_B$  or  $y_B = n_B / \sum_i n_i$ . The mole fraction is a dimensionless number.

### *State functions*

In stating energy quantities associated with the state functions of a substance, care should be taken to use the precisely defined functions and the recommended symbols, internal energy ( $U$ ), enthalpy ( $H$ ), Helmholtz energy ( $A$ ), Gibbs energy ( $G$ ), entropy ( $S$ ), or heat capacity ( $C$ ).

The term heat should be avoided in reference to state thermodynamic functions. Thus enthalpy is preferable to heat content and enthalpy of formation is preferable to heat of formation. An exception to this restriction is heat capacity for which no alternative has been accepted. The term specific heat capacity should refer only to heat capacity per unit mass. The term specific heat should be avoided. The corresponding molar quantity is molar heat capacity.

The term free energy is ambiguous and should not be used. Proper terms are Helmholtz energy or Helmholtz function and Gibbs energy or Gibbs function, symbols  $A$  and  $G$ , respectively, and referring to  $U - TS$  or  $H - TS$ , respectively. The

extensive quantities have the unit joule (J). The specific quantities have the unit joule per kilogram ( $\text{J} \cdot \text{kg}^{-1}$ ). The molar quantities have the unit joule per mole ( $\text{J} \cdot \text{mol}^{-1}$ ). Entropy, symbol  $S$ , an extensive quantity, has the unit joule per kelvin ( $\text{J} \cdot \text{K}^{-1}$ ); the specific entropy has the unit joule per kilogram kelvin ( $\text{J} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}$ ); the molar entropy has the unit joule per mole kelvin ( $\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ).

### *Equilibrium constants*

Ideally the thermodynamic equilibrium constant, based on activities of the reacting components, should be reported. In the case of most biochemical studies, however, this is not practicable. Therefore, the recommendations made in the preceding section of this document regarding the calculation and reporting of equilibrium constants and Gibbs energy changes should be observed. In accordance with the general recommendation given above, that symbols for physical quantities be differentiated from symbols for units, the symbol  $K$  should be in italic (sloping) type in printed documents, or underlined in typewritten documents, to differentiate it from the symbol for kelvin,  $K$ , which should be in roman (upright) type.

### *Electrode measurements*

Conventions concerning the signs of electric potential differences, electromotive forces, and electrode potentials, are given in Ref. 4 (Chapter 9) and are sometimes called the "Stockholm Convention" of 1953. The author should strictly adhere to them.

By these conventions the direction of a written chemical reaction, the order of representation of the corresponding electrolytic cell elements, and the assignments of sign to the electromotive force,  $E$ , of the cell obey the following consistency relationships.

1. The cell reactions and the cell diagram are written from left to right in such a way that *when* reaction occurs in this direction positive electric charge flows in the cell from left to right. The electric potential difference is equal in sign and magnitude to the electric potential of the electrode on the right minus that of the electrode on the left.

2. The electrode potential of an electrode (half-cell) is the electromotive force of a cell in which the electrode on the right is the electrode in question and the electrode on the left is a standard hydrogen electrode. A more positive electromotive force indicates a greater oxidizing potential.

### *Summary tables*

Table I lists the thermodynamic functions found in the recommendations of the main text, gives their units and a few relationships involving them. It also summarizes some of the principal recommended conditions and practices. Table II gives some SI units and their symbols.

The units of molar enthalpy change and molar Gibbs energy change are generally expressed in joules per mole ( $\text{J} \cdot \text{mol}^{-1}$ ) or kilojoules per mole ( $\text{kJ} \cdot \text{mol}^{-1}$ ) of a reaction shown, but the units should be stated in every case. For particular purposes specific enthalpy change or specific Gibbs energy change may be given per unit mass of a given reactant or product (joules per kilogram) ( $\text{J} \cdot \text{kg}^{-1}$ ).

The units of entropy change are generally expressed in joules per mole kelvin

$\cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ) for a particular process, but the units should be stated in every case. For particular purposes specific entropy change may be given per unit mass of a given reactant or product, joule per kilogram kelvin ( $\text{J} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}$ ), or the total entropy change for a stated process in which case the units are joules per kelvin ( $\cdot \text{K}^{-1}$ ).

#### TABLE I(a)

#### THERMODYNAMIC TERMS USED IN THESE RECOMMENDATIONS

]  (pH = x; etc.)	concentration of A
	activity coefficient of substance B (concentration basis)
	relative activity of solute B (Note: $a_B = \gamma_B \cdot [B]$ )
	ionic strength ( $I_c = \frac{1}{2} \sum_1^S c_i z_i^2$ ; $I_m = \frac{1}{2} \sum_1^S m_i \cdot z_i^2$ ) where $c_i$ is the concentration of the $i$ th ion, $m_i$ is the molality of the $i$ th ion, and $z_i$ is the charge number of the $i$ th ion. $S$ is the number of ion types present.
	pH-independent proper product of equilibrium concentrations (concentration equilibrium constant)
$\gamma_p$ $\gamma_p$ $\gamma_{sd}$ $\gamma_c^0$	thermodynamic equilibrium constant
	pH-dependent apparent proper product of summed equilibrium concentrations, constrained with respect to concentrations of stated species (apparent equilibrium constant)
	see $K'$
	see $K'$
	see $K'$
$\gamma_c^{0'}$ (pH = x)  $\gamma_{exp}^0$ $\gamma_{app}^0$ $\gamma$	standard Gibbs energy change corresponding to the pH-independent product of equilibrium concentrations ( $\Delta G_c^0 = -RT \ln K_c$ )
	Apparent standard Gibbs energy change corresponding to the apparent product of equilibrium concentrations at fixed pH in a buffered solution. ( $\Delta G_c^{0'} = -RT \ln K_c$ )
	see $\Delta G^{0'}$
	see $\Delta G^{0'}$
	formerly common symbol for free energy change. Now more properly called Gibbs energy change and given the symbol $\Delta G$ .
$\Delta F$ $\Delta G^0$ $\Delta G^{0'}$ $\Delta H$ $C_p$	see $\Delta F$ , $\Delta G^0$ , $\Delta G^{0'}$
	entropy change
	enthalpy change
	enthalpy change
	heat capacity at constant pressure

#### TABLE I(b)

#### SUMMARY OF THERMODYNAMIC SYMBOLS AND OTHER RECOMMENDATIONS

Quantity	Apparent value at pH = constant (x)	pH-independent value
Equilibrium constant*	$K'_c$	$K_c$
Standard molar Gibbs energy change**	$\Delta G^{0'}$ (pH = x)	$\Delta G^0$
Concentration of reactant A	$[A]_{\text{total}}$	$[A]$

\* Approximately constant proper quotient of equilibrium concentrations.  
 \*\* Formally calculated from the equilibrium constant.

# RECOMMENDED CONVENTIONS CONCERNING FACTORS IN EXPRESSIONS FOR EQUILIBRIUM CONSTANTS.

In cases where water occurs as a reactant or product, state whether the factor for water is taken as unity or as 55.5 or other number.

State whether  $10^{-\text{pH}}$  or some other measure is used for the hydrogen ion factor. The pH is not uncontested as an accurate measure of concentration or activity of the hydrogen ion.

## RECOMMENDED MEASUREMENT CONDITIONS

Temperature $t$ (or $T$ )	Primary conditions 25 °C (298.15 K) (also, vary $t$ )	Secondary conditions 37 °C (310.15 K) (also, vary $t$ )
Ionic strength, $I/\text{mol} \cdot \text{dm}^{-3}$	0.1 (made up with KCl)	0.1 (made up with KCl)
Hydrogen ion concentration	pH = 7	
Buffer concentration	lowest effective	

TABLE II

## PHYSICAL QUANTITIES, SI UNITS, AND THEIR SYMBOLS

	Name of unit	Symbol for unit			
(a) Base units					
Length	metre	m			
Mass	kilogram	kg			
Time	second	s			
Electrical current	ampere	A			
Temperature	kelvin	K			
Amount of substance	mole	mol			
Luminous intensity	candela	cd			
(b) Derived units (examples)					
Force	newton	N		(kg · m · s <sup>-2</sup> )	
Pressure	pascal	Pa		(N · m <sup>-2</sup> )	
Energy	joule	J		(kg · m <sup>2</sup> · s <sup>-2</sup> )	
Power	watt	W		(J · s <sup>-1</sup> )	
Electric charge	coulomb	C		(A · s)	
Electric potential difference	volt	V		(J · A <sup>-1</sup> · s <sup>-1</sup> )	
Electric resistance	ohm	Ω		(V · A <sup>-1</sup> )	
Frequency	hertz	Hz		(s <sup>-1</sup> )	
Area	square metre	m <sup>2</sup>			
Volume	cubic metre	m <sup>3</sup>			
Density	kilogram per cubic metre	kg · m <sup>-3</sup>			
(c) Prefixes					
Multiplier	Prefix	Symbol	Multiplier	Prefix	Symbol
10 <sup>-1</sup>	deci	d	10	deca	da
10 <sup>-2</sup>	centi	c	10 <sup>2</sup>	hecto	h
10 <sup>-3</sup>	milli	m	10 <sup>3</sup>	kilo	k
10 <sup>-6</sup>	micro	μ	10 <sup>6</sup>	mega	M
10 <sup>-9</sup>	nano	n	10 <sup>9</sup>	giga	G
10 <sup>-12</sup>	pico	p	10 <sup>12</sup>	tera	T
10 <sup>-15</sup>	femto	f	10 <sup>15</sup>	peta	P
10 <sup>-18</sup>	atto	a	10 <sup>18</sup>	exa	E

The units of absolute entropy and heat capacity are the same as those for entropy change ( $\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ,  $\text{J} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}$ , or  $\text{J} \cdot \text{K}^{-1}$ ) but refer to a particular substance or aggregate of substances rather than to a process.

Some thermodynamic relations: the units for these quantities are joules, J, for the extensive quantity total energy, joules per mole ( $\text{J} \cdot \text{mol}^{-1}$ ) for molar energy, and joule per kilogram ( $\text{J} \cdot \text{kg}^{-1}$ ) for specific energy.

$$\Delta U = Q + W$$

1st law of thermodynamics. The increase of internal energy of a system is the sum of heat supplied to the system and work done on the system.

$$\Delta H = \Delta U + p\Delta V$$

For a constant pressure system an increase in enthalpy of the system ( $\Delta H$ ) results in an increase in internal energy ( $\Delta U$ ) and work done by the system ( $p\Delta V$ ).

$$\Delta H = H(T_2) - H(T_1) = \int C_p dT$$

For a system at constant pressure to which energy is supplied the increase of enthalpy is the integral of  $C_p$  over the range of the temperature change it causes.

$$\Delta G = \Delta H - T\Delta S$$

The increase in Gibbs energy of a system ( $\Delta G$ ) at constant temperature is the increase in enthalpy ( $\Delta H$ ) minus the increase in  $T\Delta S$ .

## REFERENCES

- 1 Guide for the Presentation in the Primary Literature of Numerical Data Derived from Experiments. CODATA Bulletin No. 9, December 1973.
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- 4 International Union of Pure and Applied Chemistry (IUPAC). McGlashan, M. L. (1970). Manual of Symbols and Terminology for Physicochemical Quantities and Units, Butterworth and Co., London, Toronto, etc. (SBN-408 89350 8); (1970) Pure Appl. Chem. 21, No. 1, 3–44; Revision, M. A. Paul (1975) Butterworth and Co., London (ISBN 0 408 70671 6).
- 5 International Organization for Standardization (ISO). SI units and recommendations for the use of their multiples and of certain other units. International Standard ISO-1000, First edition-1973-02-01, American National Standards Institute, New York. See also, for more detail: International Standard ISO-31/0-1974 and ISO 31/I-XII (1965–1975) which deal with quantities, units, symbols, conversion factors, and conversion tables for various branches of science and technology. Copies are obtained through the ISO-member national standards organizations of various countries.
- 6 Bureau International des Poids et Mesures (BIPM): Le Système International d'Unités, 1970, OFFILIB, 49 Rue Gay-Lussac, F75 Paris 5, France. Authorized English translations are available from: (a) The International System of Units (SI), National Bureau of Standards (USA) Publication 330, 1974, United States Government Printing Office, Washington, DC. or (b) The International System of Units (SI), Her Majesty's Stationery Office, London.