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Limits to anaerobic energy and cytosolic concentration in the living cell

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For many physical systems at any given temperature, the set of all states where the system's free energy reaches its largest value can be determined from the system's constitutive equations of internal energy and entropy, once a state of that set is known. Such an approach is fraught with complications when applied to a living cell, because the cell's cytosol contains thousands of solutes, and thus thousands of state variables, which makes determination of its state impractical. We show here that, when looking for the maximum energy that the cytosol can store and release, detailed information on cytosol composition is redundant. Compatibility with cell's life requires that a single variable that represents the overall concentration of cytosol solutes must fall between defined limits, which can be determined by dehydrating and overhydrating the cell to its maximum capacity. The same limits are shown to determine, in particular, the maximum amount of free energy that a cell can supply in fast anaerobic processes, starting from any given initial state. For a typical skeletal muscle in normal physiological conditions this energy, i.e., the maximum anaerobic capacity to do work, is calculated to be about 960 J per kg of muscular mass. Such energy decreases as the overall concentration of solutes in the cytosol is increased. Similar results apply to any kind of cell. They provide an essential tool to understand and control the macroscopic response of single cells and multicellular cellular tissues alike. The applications include sport physiology, cell aging, disease produced cell damage, drug absorption capacity, to mention the most obvious ones.

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I. INTRODUCTION

The cytosol—the aqueous solution within the living cell—is the medium where most of the chemical reactions essential to a cell's life take place. It is a complex solution that contains several tens of thousands of different solutes, ranging over a multitude of ions, a host of small organic and inorganic molecules, and a great number of large organic compounds such as proteins [1–5]. A fundamental function of the cytosol is to store and release energy. In this respect, its role is not dissimilar to that of the electrolyte solution of a rechargeable electric battery. In the cytosol case, however, this function is performed by a myriad of chemical reactions between the cytosol solutes and by exchanges of materials with the environment. In spite of such complexity, we show here that the free energy of the cytosol, i.e., the available energy contained in a living cell, is determined by the overall concentration of cytosol solutes.

Essential to the present analysis is the observation that, at a finite temperature, the internal energy that can be stored in a finite volume system is finite. This fact is a direct consequence of the first law of thermodynamics. When considered in the light of the second law of thermodynamics, it implies that the specific free energy of a system (i.e., the energy that at a given temperature a system can store and spend per unit volume) cannot exceed a definite limit, which depends on the system [6]. The analysis that leads to this result is reviewed in Appendix A. When applied to the cytosol of a living cell, the same result requires that the overall concentration of solutes in the cytosol must fall within a definite interval, which is the admissible range of overall concentration of cytosol solutes, or the *cell's admissible range* for short. Outside that range, the cytosol's free energy becomes greater than the maximum amount of free energy that the cytosol can contain, which is bound to produce irreversible changes in the cell or even the cell's death.

Each cell has its own admissible range. The ends of this range are the lowest and the largest overall cytosolic concentrations that are compatible with the cell. As shown in Sec. V, a way to determine the limit concentrations of a cell is to measure the water content of its cytosol in simple experiments in which the cell is dehydrated and overhydrated to its maximum capacity. The same experiments can also be used to determine the effective concentration or *activity* of the equivalent solute.

The limits to overall cytosolic concentration established in this paper bear important consequences in cell biology. Because the cytosol contains such a large number of solutes, a limit to the maximum content of solutes implies even a tighter limit to the concentration of each solute. Adding any amount of any solute beyond that limit, therefore, must be accompanied by a reduction in the concentrations of the other solutes in order to keep the overall cytosolic concentration below the admissible limit. In particular, any solute intake or any chemical reaction that produces an increase in the cytosolic solutes may require some output of solutes from the cell or the formation of solute aggregates to keep the overall cytosolic concentration within the admissible values. For this reason, the concentration limit in question may be of relevance when studying the drug absorption capacity of the cell, or the effects of the accumulation of spurious material in the cytosol resulting from aging or disease.

A further consequence concerns cell energetics. A limit to cytosolic concentration entails a limit to the energy that a cell can store. This means, in particular, that for any state of the cell there is a limit to the energy that the cell can spend in fast anaerobic processes, i.e., when no nutrient can be taken from the surroundings. Clearly, the amount of energy stored in the cytosol is related to the capacity of a cell to

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spend energy and, thus, to the cell's vitality. This capacity depends on the state of the cell. The paper shows how to calculate it once the cytosol composition is given and the cell's dehydration and overhydration limits are known (Sec. VI). When applied to skeletal muscle cells, this result can be used to determine the work that a given mass of skeletal muscle can do in fast anaerobic excises, such as weight lifting, jumping, sprint running, etc. A practical example is presented in detail in Sec. VI. The implications for sport science and cell physiology are evident.

II. CYTOSOL FREE ENERGY

As in every physical system, the state of the cytosol is defined by its state variables. In the cytosol case, these are the state variables of any solution, namely pressure (p), absolute temperature (T), moles of solvent or water in the cytosol's case ($n_{\mathrm{H_2O}}$), and moles of solutes (n_j, with $j = 1, 2, \ldots s$, where s is the number of solutes). Even the simplest cell contains thousands of different solutes in its cytosol, which makes s in the order of 10^4. A practical way to take account of such a large number of variables—many of which may not even be known accurately—is presented in the following sections.

Volume, energy, entropy, etc., of cytosol depend on the state variables according to the well-known constitutive equations of any liquid mixture. In particular, the constitutive equation of the cytosol's Gibbs free energy G can be expressed in the form (see Appendix B)

$$G = n_{\mathrm{H_2O}}\mu^o_{\mathrm{H_2O}}(p^o,T) + \sum_{j=1}^{s} n_j\mu^o_j(p^o,T) + V\Delta p + RT\left[n_{\mathrm{H_2O}} \ln \frac{\gamma_{\mathrm{H_2O}} n_{\mathrm{H_2O}}}{n_{\mathrm{H_2O}} + \sum_{j=1}^{s} n_j} + \sum_{j=1}^{s} n_j \ln \frac{\gamma_j n_j}{m_{\mathrm{H_2O}}} \right] + C. \tag{2.1}$$

In this equation, $\mu^o_{\mathrm{H_2O}}$ and μ^o_j denote the chemical potentials of solvent and solutes in a reference state at temperature T and pressure p°. The quantity $\Delta p = p - p^\circ$ represents the excess of p over the reference pressure, while V is the cytosol volume and $m_{\mathrm{H_2O}}$ is the mass of solvent. The dimensionless factors $\gamma_{\mathrm{H_2O}}$ and γ_j are the activity coefficients of solvent and solutes, respectively. Finally, R is the universal gas constant and C is an arbitrary constant that depends on the reference value assumed for the internal energy of the solution. The above notation is almost standard and is the same as that adopted in [6]. The nondimensionless ratio $n_j/m_{\mathrm{H_2O}}$ appearing in the arguments of the logarithms under summation in the above equation originates from expressing the concentration of the solutes in molalities (moles per kg of solvent). Thus, in Eq. (2.1) and the ones that follow from it, $m_{\mathrm{H_2O}}$ must be understood to be measured in kg. According to the usual convention, the same logarithm arguments are understood to be divided by 1 mol/kg to make them dimensionless.

Water makes up more than 70% of the total weight of the cytosol. The remaining 30% weight is shared by a myriad of solutes. Therefore, the concentration of each solute is quite low. Most of the solutes are organic compounds of molecular weight that easily exceeds ten times the molecular weight of water. This makes the mole number of each solute quite small, so that the quantities n_j are mostly in the range 10^{-3} or less. Given that $\gamma_{\mathrm{H_2O}}$ is not far from unity, the value of the argument of the first logarithm in the brackets of Eq. (2.1) is approximately equal to 1 as a result. Moreover, the same argument is not subjected to undergo significant changes, since the overall amount of solutes does not vary much during the cell's life. As a result, the logarithm in question is quite small and almost constant. At constant temperature, therefore, no sensible error is made if the contribution to G from the said logarithm is ignored or considered as included in C. Accordingly, we shall henceforth refer to the following approximate expression of the cytosol's Gibbs free energy:

$$G = n_{\mathrm{H_2O}}\mu^o_{\mathrm{H_2O}}(p^o,T) + \sum_{j=1}^{s} n_j\mu^o_j(p^o,T) + RT\sum_{j=1}^{s} n_j \ln \frac{\gamma_j n_j}{m_{\mathrm{H_2O}}} + V\Delta p + C. \tag{2.2}$$

The cytosol's Helmholtz free energy Ψ can accordingly be expressed as

$$\Psi = n_{\mathrm{H_2O}}\mu^o_{\mathrm{H_2O}}(p^o,T) + \sum_{j=1}^{s} n_j\mu^o_j(p^o,T) + RT\sum_{j=1}^{s} n_j \ln \frac{\gamma_j n_j}{m_{\mathrm{H_2O}}} - p^o V + C \tag{2.3}$$

as readily follows from Eq. (2.1) and the definition of Ψ :

$$\Psi = G - pV. \tag{2.4}$$

Not all the free energy (2.3) is subjected to a thermodynamic limit. As discussed in Appendix B, the part of Ψ that determines the admissible range of the cell is given by

$$\Psi'' = RT\sum_{j=1}^{s} n_j \ln \frac{\gamma_j n_j}{m_{\mathrm{H_2O}}} - p^o V, \tag{2.5}$$

which may well represent a tiny, though important, part of the total free energy of the cytosol.

In seeking the cell's admissible range, it is convenient to refer to the quantity $\bar{\Psi}''$ representing the specific value of Ψ'' per mole of solvent. By dividing both sides of Eq. (2.5) by $n_{\mathrm{H_2O}}$ we obtain

$$\bar{\Psi}'' = RT\sum_{j=1}^{s} \frac{n_j}{n_{\mathrm{H_2O}}} \ln \frac{\gamma_j n_j}{n_{\mathrm{H_2O}} M_{\mathrm{H_2O}}} - p^o \bar{V}, \tag{2.6}$$

where $M_{\mathrm{H_2O}} = 18.015 \times 10^{-3}$ Kg/mol is the molar mass of water, while $\bar{V}$ is the cytosol volume per mole of solvent, i.e.,

$$\bar{V} = \frac{V}{n_{\mathrm{H_2O}}}. \tag{2.7}$$

If $\bar{\Psi}''_{\text{max}}$ denotes the maximum value that $\bar{\Psi}''$ can attain at a given temperature T, we have that, at the same temperature,

$$\bar{\Psi}'' \leqslant \bar{\Psi}''_{\text{max}}. \tag{2.8}$$

This relation defines the cell's admissible range in space of variables n_{H_2O} and n_j, because $\bar{\Psi}'' = \bar{\Psi}''(n_{H_2O}, n_j, T)$. From Eqs. (2.6) and (2.7), the same range can be expressed more explicitly as

$$RT \sum_{j=1}^{s} \frac{n_j}{n_{H_2O}} \ln \frac{\gamma_j n_j}{n_{H_2O} M_{H_2O}} - p^o \bar{V} \leqslant \bar{\Psi}''_{\text{max}}. \tag{2.9}$$

The cell's limit surface is the boundary of this range. That is,

$$RT \sum_{j=1}^{s} \frac{n_j}{n_{H_2O}} \ln \frac{\gamma_j n_j}{n_{H_2O} M_{H_2O}} - p^o \bar{V} = \bar{\Psi}''_{\text{max}}. \tag{2.10}$$

III. EQUIVALENT SOLUTE

As they stand, relations (2.6), (2.9), and (2.10) are hardly practical due to the large number of terms implied by the summation appearing in them (remember that s is in the order of 10^4). It is fortunate that, for processes that occur at constant temperature and pressure, that summation can be reduced to just one single term according to the formula,

$$\sum_{j=1}^{s} \frac{n_j}{n_{H_2O}} \ln \frac{\gamma_j n_j}{n_{H_2O} M_{H_2O}} = \frac{n_{\text{eq}}}{n_{H_2O}} \ln \frac{\gamma_{\text{eq}} n_{\text{eq}}}{n_{H_2O} M_{H_2O}}. \tag{3.1}$$

In this formula, n_{eq} indicates the overall moles of solutes contained in volume V of cytosol, i.e.,

$$n_{\text{eq}} = \sum_{j=1}^{s} n_j, \tag{3.2}$$

while n_{H_2O} are the moles of solvent contained in the same volume and γ_{eq} is defined by

$$\gamma_{\text{eq}} = \frac{M_{H_2O}}{n_{\text{eq}}} \left[\prod_{j=1}^{s} \left(\frac{\gamma_j n_j}{M_{H_2O}} \right)^{n_j} \right]^{1/n_{\text{eq}}}. \tag{3.3}$$

The symbol Π appearing in this equation indicates the product of a sequence, i.e.,

$$\prod_{i=1}^{k} y_i = y_1 y_2 \ldots y_k. \tag{3.4}$$

To prove Eq. (3.1), it is convenient to adopt the following notation:

$$a = n_{\text{eq}} = \sum_j n_j, \quad a_i = n_i, \quad b = \frac{\gamma_{\text{eq}} n_{\text{eq}}}{M_{H_2O}},$$
$$b_i = \frac{\gamma_i n_i}{M_{H_2O}}, \quad \text{and} \quad x = n_{H_2O}. \tag{3.5}$$

Thus, Eq. (3.1) can be written in the formally simpler form

$$\sum_{j=1}^{s} \frac{a_j}{x} \ln \frac{b_j}{x} = \frac{a}{x} \ln \frac{b}{x}. \tag{3.6}$$

It can then be observed that the following equations hold true for any value of x:

$$\begin{aligned} \sum_{j=1}^{s} \frac{a_j}{x} \ln \frac{b_j}{x} &= \frac{a_1}{x} \ln \frac{b_1}{x} + \frac{a_2}{x} \ln \frac{b_2}{x} + \cdots + \frac{a_s}{x} \ln \frac{b_s}{x} \\ &= \frac{a_1}{x} \ln b_1 - \frac{a_1}{x} \ln x + \frac{a_2}{x} \ln b_2 - \frac{a_2}{x} \ln x \\ &\quad + \cdots + \frac{a_s}{x} \ln b_s - \frac{a_s}{x} \ln x \\ &= \frac{1}{x} \ln \left(b_1{}^{a_1} b_2{}^{a_2} \ldots b_s{}^{a_s} \right) \\ &\quad - \frac{1}{x} (a_1 + a_2 + \cdots + a_s) \ln x. \end{aligned} \tag{3.7}$$

The validity of Eq. (3.6) follows from the last of these equations once we set

$$a = a_1 + a_2 + \cdots + a_s \tag{3.8}$$

and

$$b^a = b_1{}^{a_1} b_2{}^{a_2} \ldots b_s{}^{a_s} = \prod_{j=1}^{s} b_j{}^{a_j}, \tag{3.9}$$

respectively. As Eqs. (3.5), (3.8), and (3.9) should make it apparent, Eq. (3.6) is nothing but Eq. (3.1) in a different notation. The proof is thus completed.

IV. A SIMPLER EXPRESSION FOR THE LIVING CELL'S LIMIT SURFACE

Thanks to Eq. (3.1), much of the information contained in relation (2.9) can be obtained from the simpler relation

$$RT \frac{n_{\text{eq}}}{n_{H_2O}} \ln \frac{\gamma_{\text{eq}} n_{\text{eq}}}{n_{H_2O} M_{H_2O}} - p^o \bar{V} \leqslant \bar{\Psi}''_{\text{max}}, \tag{4.1}$$

which follows from relations (2.9) and (3.1). Relation (4.1) shows that the quantity n_{eq} plays the role of one single solute that, as far as the cell's admissible range is concerned, is *equivalent* to all the solutes which are actually contained in the cytosol. The index "eq" is appended to n to remind us of this fact. The quantity γ_{eq} is an appropriate function of n_{eq} and n_{H_2O}, which may be interpreted as the activity coefficient of such an equivalent solute.

Relation (4.1) holds true, in particular, when hydration and dehydration processes at constant temperature and pressure are considered. In these processes, the variable n_{H_2O} changes while most of the variables n_j remain constant. In the living cell, the values of n_{eq} and γ_{eq} may generally change with n_{H_2O}. However, for hydration and dehydration processes that involve comparatively small changes in the cytosol's water content—which generally is the case of the processes considered in this paper—the dependence of n_{eq} and γ_{eq} on n_{H_2O} may be ignored. In this case, the values of n_{eq} and γ_{eq} can be taken as coinciding with those that the same variables assume at the beginning of the process.

Strictly speaking, also $\bar{V}$ depends on n_{H_2O}, as apparent from Eq. (2.7) and from the fact that V varies with n_{H_2O}. In practice, however, the water content of the cytosol is so high with respect to that of the solutes that the dependence of $\bar{V}$ from n_{H_2O} can be ignored. In these conditions, during the above hydration or dehydration process $\bar{V}$ is almost constant and very near to the molar volume of water.

By taking the equality sign in relation (4.1), we obtain the cell's limit surface:

$$RT\frac{n_{\rm eq}}{n_{\rm H_2O}}\ln\frac{\gamma_{\rm eq}n_{\rm eq}}{n_{\rm H_2O}M_{\rm H_2O}} - p^o\bar{V} = \bar{\Psi}''_{\max}. \tag{4.2}$$

In plane ($n_{\rm eq}$, $n_{\rm H_2O}$), the cell's admissible range is represented by the region of points [$n_{\rm eq}$, $n_{\rm H_2O}$] that satisfy relation (4.1). In the same plane, the cell's limit surface reduces to curve (4.2) that delimits this region. For processes where $n_{\rm eq}$ is a function of $n_{\rm H_2O}$, the pairs $n_{\rm eq}$ and $n_{\rm H_2O}$ describe a line in the above region, while Eq. (4.2) defines the end points of that line.

V. EXPERIMENTAL DETERMINATION OF THE CELL'S ADMISSIBLE RANGE

At constant temperature and pressure, a living cell can progressively be dehydrated by immersing it into a hypertonic solution. As the cell loses water by osmosis, it shrinks and eventually dies. Starting from any given state of the cell, let w_d be the smallest value of $n_{\rm H_2O}$ that the cell can tolerate without suffering permanent changes. If not dehydrated below the value w_d, the cell is capable of recovering its original state and functions when subsequently rehydrated, which indicates that the dehydration process took place entirely within the admissible range of the cell. On the contrary, if the cell is dehydrated beyond limit w_d, it will suffer permanent damage. In this case, subsequent rehydration to the original water content will not bring the cell back to its original state, because dehydration beyond the cell's admissible range produces permanent changes in the cell's response and hence its constitutive equations.

Because it is a state at the boundary of the cell's admissible range, the state of the cell at the limit of dehydration belongs to cell's limit surface. Thus, $\bar{\Psi}'' = \bar{\Psi}''_{\max}$ if $n_{\rm H_2O} = w_d$. Therefore, from Eq. (4.1) we have

$$\bar{\Psi}''(w_d) = RT\frac{n_{\rm eq}}{w_d}\ln\frac{\gamma_{\rm eq}n_{\rm eq}}{w_d M_{\rm H_2O}} - p^o\bar{V}|_d = \bar{\Psi}''_{\max}. \tag{5.1}$$

An analogous situation occurs when the cell is overhydrated. This can be done by immersing the cell into a hypotonic solution, rather than a hypertonic one as in the previous case. The cell absorbs water from its surroundings by osmosis and it swells. Again if the process lasts long enough, the cell may die as a result. Let w_h be the largest value of $n_{\rm H_2O}$ that the cell can maintain under isothermal, constant pressure conditions without suffering permanent changes. Similarly to what applies to the dehydration limit, the limit state of hydration must belong to the cell's limit surface. Thus, $\bar{\Psi}'' = \bar{\Psi}''_{\max}$ also for $n_{\rm H_2O} = w_h$. From Eq. (4.1) we have, therefore,

$$\bar{\Psi}''(w_h) = RT\frac{n_{\rm eq}}{w_h}\ln\frac{\gamma_{\rm eq}n_{\rm eq}}{w_h M_{\rm H_2O}} - p^o\bar{V}|_h = \bar{\Psi}''_{\max}. \tag{5.2}$$

About 99% of cytosol molecules are water [1–5], which makes $n_{\rm H_2O}$ much greater than $n_{\rm eq}$ and, *a fortiori*, much greater than the moles n_j of any of the cytosol solutes. As a consequence, the cytosol volume per mole of solvent, $\bar{V}$, approximates the molar volume of water and it does not change appreciably unless the changes in $n_{\rm H_2O}$ are quite large. Because living cells can only withstand modest changes in their water content, it is reasonable to assume that in the above hydration and dehydration processes $\bar{V}|_h \approx \bar{V}|_d$. Then, from Eqs. (5.1) and (5.2) it is not difficult to conclude that, to a good approximation,

$$\gamma_{\rm eq} = \frac{M_{\rm H_2O}}{n_{\rm eq}}\left(\frac{w_d{}^{w_h}}{w_h{}^{w_d}}\right)^{1/w_h - w_d} \tag{5.3}$$

Based on this result, the activity $\gamma_{\rm eq}$ can be determined experimentally as follows. Starting from any given initial state of the cell, the values of w_h and w_d relevant to that initial state are determined experimentally through a sequence of processes in which the cell is hydrated and dehydrated as appropriate. The value of $n_{\rm eq}$ is calculated from Eq. (3.2) once the quantities $n_{\rm i}$ are obtained from a chemical analysis of the cell's cytosol in the considered initial state. The factor $\gamma_{\rm eq}$ can then be calculated from Eq. (5.3), since all the variables entering this equation are now known. Strictly speaking, the value of $\gamma_{\rm eq}$ depends on the initial state of the cell, because so do the quantities w_h, w_d, and $n_{\rm eq}$. However, in the range of states that are accessible to a living cell, these quantities do not suffer much change. This implies even smaller changes in $\gamma_{\rm eq}$, according to Eq. (5.3). For this reason, in the absence of more precise data, $\gamma_{\rm eq}$ will be treated as a constant.

As apparent from Eq. (4.1), the variables $\gamma_{\rm eq}$ and $n_{\rm eq}$ determine the function $\bar{\Psi}'' = \bar{\Psi}''(n_{\rm H_2O})$ completely. The same variables also determine $\bar{\Psi}''_{\max}$ through Eqs. (2.10) or (4.2), once w_h and w_d are known. The cell's admissible range (5.1) and limit surface (5.2) are thus determined by w_h, w_d, and $n_{\rm eq}$, thanks to Eq. (5.3).

To exemplify, let us refer to a typical skeletal muscle cell of a generic living being. Let the cell be in a given relaxed state at normal body temperature $T = 310$, $K = 37\,^\circ$C, which we take as the cell's initial state. In this state, the cell's water content is assumed to be 70% of the cell wet weight. As recalled above, water molecules account for about 99% of all molecules in the cell. Thus, if $n^o_{\rm H_2O}$ is the number of water moles in the cytosol in the initial state, the value of $n_{\rm eq}$ in that state is, to a good approximation, $n^o_{\rm eq} = 0.01 n^o_{\rm H_2O}$. Of course, $n^o_{\rm eq}$ can be determined more precisely from a quantitative chemical analysis of the cytosol. Suppose then that the cell's dehydration and overhydration limits are $w_d = 0.8 n^o_{\rm H_2O}$ and $w_h = 1.1 n^o_{\rm H_2O}$, respectively. These limits correspond to water content of 56% and 82% of the total cell weight, respectively, and are consistent with the values reported in the literature for a skeletal muscle cell (see, e.g., [7–9]). Inserted into Eq. (5.3), these values of w_d and w_h yield $\gamma_{\rm eq} = 0.6165$. The initial amount of cytosol can be taken arbitrarily. Thus, no restriction arises if we take $n^o_{\rm H_2O} = 1$ mol, which we shall henceforth assume. A plot of $\bar{\Psi}'' = \bar{\Psi}''(n_{\rm H_2O})$ for $\gamma_{\rm eq} = 0.6165$ and $n_{\rm eq} = n^o_{\rm eq} = 0.01$ mol is presented in Fig. 1. For the considered cell, the limit value $\bar{\Psi}''_{\max}$ as calculated from Eqs. (5.1) or (5.2) is $\bar{\Psi}''_{\max} = -\,29.08$ J/mol. (Remember that $\bar{\Psi}''$ represents an energy per mole of solvent, water in the present case.) The minimum of $\bar{\Psi}''$ is reached for $n_{\rm H_2O} = w_{\rm m} = 0.93$ mol and is $\bar{\Psi}''_{\min} = -\,29.51$ J/mol. This minimum, however, plays no role in what follows. It is unrelated to the minimum of the cytosol free energy Ψ, because Ψ'' and thus its specific value

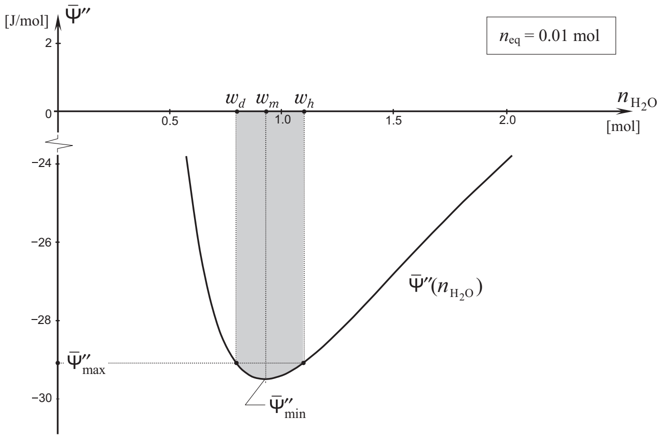

FIG. 1. Plot of $\bar{\Psi}'' = \bar{\Psi}''(n_{H_2O})$ as obtained from Eq. (4.1) for $\gamma_{eq} = 0.6165$ and $n_{eq} = 0.01$ mol. All values of n_{H_2O} within the interval $[w_d, w_h]$ are admissible for the cell, according to Eq. (4.1). At the ends of that interval, $\bar{\Psi}''$ equals $\bar{\Psi}''_{max}$. Normal body temperature $T = 310\,\text{K} = 37\,^\circ\text{C}$ is assumed.

$\bar{\Psi}''$ are only a small part of Ψ [cf. definitions (2.5) and (2.6) and Appendix B]. The reader is referred to [6] for a complete treatment of this point.

Relation (4.1) sets limits to n_{eq}, that is to overall concentration of cytosol solutes. As observed, these limits cannot be exceeded without producing irreversible changes in the cell. Figure 2 represents the diagram of $\bar{\Psi}''$ vs n_{eq}/n_{H_2O} as obtained from Eq. (4.1) for the skeletal muscle cell considered in the present example. The quantity $\bar{V}$ is assumed to be equal to the molar volume of water. The admissible range of overall cytosolic concentrations (n_{eq}/n_{H_2O}) correspond to the part of the diagram that lies below the line $\bar{\Psi}'' = \bar{\Psi}''_{max}$. The maximum and minimum values of n_{eq} per mole of cytosolic water are reached at the ends of this range. In the considered example, these values turn out to be $n_{eq}|_{max} = 12.70 \times 10^{-3}$ mol and $n_{eq}|_{min} = 8.92 \times 10^{-3}$ mol, per mole of H_2O.

VI. MAXIMUM CAPACITY OF ANAEROBIC ENERGY PRODUCTION

Muscle and nervous cells must often release comparatively large amounts of energy in such a short time that there is no time for them to exchange material with the surroundings. In this case the energy is taken directly by hydrolysis of the adenosine triphosphate (ATP) contained of the cell's cytosol. The reaction is best written as [10]

$$\text{ATP}^{4-}(\text{aq}) + \text{H}_2\text{O} \rightarrow \text{ADP}^{3-}(\text{aq}) + P_\text{i}{}^{2-}(\text{aq}) + \text{H}_3\text{O}^+(\text{aq}), \tag{6.1}$$

and it produces adenosine diphosphate (ADP), some inorganic phosphate group (P_i^{2-}), and hydronium ions (H_3O^+). In normal physiological conditions of concentration and temperature, this reaction liberates some 50 kJ per mole of ATP [11].

To produce enough energy from its limited volume, the cell keeps replacing the ATP molecules of the cytosol as soon as they are consumed. It does so through a chain of very fast reactions which produces ATP from the cytosol's reserves of phosphocreatine and glycogen [12,13]. When any exchange of material with the surroundings is prevented, these ATP producing reactions are anaerobic and they do not result in a net change in the overall number of molecules dissolved in the cytosol. By contrast, the hydrolysis of ATP adds two new moles of solutes to the cytosol for each mole of ATP that is transformed into ADP, as Eq. (6.1) makes it apparent. Thus, when the cell cannot exchange material with the surroundings, anaerobic energy production results in an increase in the overall concentration of cytosol solutes. This entails a limit to the maximum amount of energy that a cell can produce in fast anaerobic processes, due to the limit to the maximum value n_{eq}/n_{H_2O} that the cell can bear.

Starting from a given initial state, let $\Delta n_{eq}|_{max}$ be the maximum amount of solutes that can be added to the cytosol without producing permanent changes in the cell. Since the ratio n_{eq}/n_{H_2O} cannot exceed the value $(n_{eq}/n_{H_2O})_{max}$, we have that

$$\Delta n_{eq}|_{max} = \left(\frac{n_{eq}}{n_{H_2O}}\right)_{max} n_{H_2O} - \frac{n^o_{eq}}{n^o_{H_2O}} n^o_{H_2O}, \tag{6.2}$$

where n_{H_2O} is the water content of the cytosol in the final state. If the cell does not exchange materials with its surroundings, the cytosol water content barely changes, because water is by far the largest component of the cytosol. In these conditions, by referring to one mole of cytosolic water, we have that $n_{H_2O} = n^o_{H_2O} = 1$ mol. The above equation can then be written as

$$\Delta n_{eq}|_{max} = n_{eq}|_{max} - n^o_{eq}. \tag{6.3}$$

All quantities in this equation are understood to be per mole of cytosolic water.

The maximum number of moles of ATP that the cell can hydrolyze in fast anaerobic processes is one–half $\Delta n_{eq}|_{max}$, since two molecules of solute are added to the cytosol per ATP molecule that is hydrolyzed. Therefore, the maximum amount of energy that the cell can produce per mole of cytosolic water in a fast anaerobic process is

$$\bar{\psi}_{max} = \tfrac{1}{2}\Delta_r G \Delta n_{eq}|_{max}\,(\text{kJ/mol}), \tag{6.4}$$

where $\Delta_r G$ is the appropriate reaction energy, i.e., the energy liberated by reaction (6.1) per mole of ATP. In normal physiological conditions, $\Delta_r G \cong 50$ kJ/mol, as mentioned above. (This may actually be a conservative estimate, as suggested in a recent review by Barclay [14].)

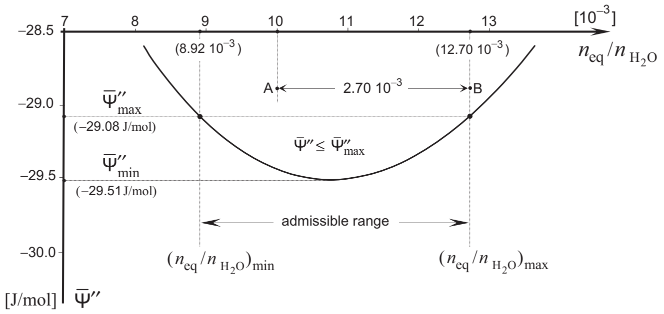

FIG. 2. Admissible range of overall solute concentration per mole of cytosolic water as obtained from limitation (2.8) for the cell considered in the diagram in Fig. 1. At the considered temperature and pressure, the admissible values of the ratio n_{eq}/n_{H_2O} range from 8.92×10^{-3} to 12.70×10^{-3}.

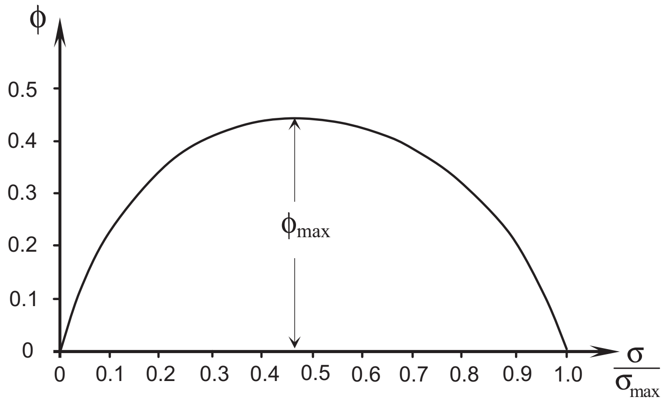

FIG. 3. Typical bell-shaped curve representing muscle efficiency as a function of $\sigma/\sigma_{\max}$, as obtained from the semiempirical formula by Hill [18].

When contracting fast, a muscle does work at the expenses of the chemical energy of the ATP that it contains. The ratio of work done to the free energy liberated from ATP hydrolysis is variously referred to as thermodynamic efficiency [15], chemiomechanical efficiency [16], contraction coupling efficiency [17], to mention just some of the terms in use. We shall denote this ratio by the symbol ϕ and refer to it simply as *efficiency*. Denoting by ϖ the mechanical work done by the muscle per mole of ATP consumed, we have that

$$\phi = \frac{\varpi}{\Delta_r G}. \tag{6.5}$$

A vast literature starting from the pioneering work by Hill [18] indicates that efficiency of muscle contraction depends on the ratio $\sigma/\sigma_{\max}$ of the applied stress σ (force per unit area of muscle fiber cross section) over the maximum isometric stress $\sigma_{\max}$. The latter stress is a characteristic constant of the cell and its value depends on the kind of muscle under consideration. The relation between ϕ and $\sigma/\sigma_{\max}$ is a bell-shaped curve such as the one represented in Fig. 3 (cf., e.g., [16,19,20]). Efficiency goes to zero for $\sigma/\sigma_{\max} = 0$ (no stress is applied to the muscle) and $\sigma/\sigma_{\max} = 1$ (isometric condition, no muscle contraction). In the vast majority of cases, maximum efficiency is reached for $\sigma/\sigma_{\max} \cong 0.5$. The value of maximum efficiency ranges from around 0.2 to around 0.6, depending on animal species, muscle kind, and whether reference to *in vivo* or *in vitro* values is made. A comparatively large *in vivo* value of $\phi = 0.68$ was found by Jubrias and co-workers for the first dorsal intraosseus muscle of the human hand [17,21]. In what follows we shall generally refer to mammal, bird, and frog muscles and we shall assume $\phi_{\max} = 0.4$. This is somehow larger than the value of 0.25–0.3 which may be regarded as more realistic (cf., e.g., [14,22]). However, the present larger value of $\phi_{\max}$ is adopted here both in view of our conservative estimate of $\Delta_r G$, and because the velocity of the anaerobic contractions we are considering is bound to limit the extent of ion pumping across the sarcolemma, thus increasing the efficiency of muscle contraction.

By multiplying Eq. (6.4) by $\phi_{\max}$, we obtain the maximum amount of mechanical work that a muscle cell can release per mole of cytosolic water in a fast anaerobic process:

$$\bar{w}_{\max} = \tfrac{1}{2}\phi_{\max}\Delta_r G \Delta n_{\text{eq}}\big|_{\max}\ (\text{kJ/mol}). \tag{6.6}$$

In skeletal muscles, intracellular or cytosolic water accounts for about 0.64 of the wet weight of the muscle tissue, cf., e.g., [23]. Therefore, the number of moles of cytosolic water per kilogram of muscle tissue can be expressed as

$$\bar{n}_{cw} = 0.64\frac{1}{M_{\text{H}_2\text{O}}} = 35.52\,(\text{mol/kg}), \tag{6.7}$$

where $M_{\text{H}_2\text{O}}$ is the molar mass of water already introduced in Eq. (2.6). In view of Eq. (6.6), the maximum work that can be released per kg of muscle tissue in a fast anaerobic process is thus given by

$$w_{\max} = 35.52\bar{w}_{\max}\ (\text{kJ/kg}). \tag{6.8}$$

Let us apply Eqs. (6.6) and (6.8) to find the maximum work that can be supplied by a muscular tissue undergoing a fast anaerobic contraction starting from an initial condition in which $n_{\text{eq}}/n_{\text{H}_2\text{O}} = 0.01$. From eq. (6.3) and from the data obtained in the previous section, we calculate $\Delta n_{\text{eq}}|_{\max} = 2.7 \times 10^{-3}$ mol per mole of cytosolic water (segment *AB* of Fig. 2). Therefore, by assuming $\Delta_r G = 50$ kJ/mol and $\phi_{\max} = 0.4$ and by applying Eq. (6.6), we obtain $\bar{w}_{\max} = 27$ J/mol. Thus, from Eq. (6.8) the maximum work that the muscle can produce in a fast anaerobic contraction turns out to be $w_{\max} = 959$ J/kg, per kilogram of wet tissue. If used in weight lifting, 1 kg of this muscle could lift, say, a 95.9-kg weight to 1 m or a 191.8-kg weight to 0.5 m, to mention just two possibilities.

It may help to compare this result it with a rough estimate made in more traditional and simpler ways. Assume, for instance, that anaerobic work is derived from just ATP and phosphocreatine (PCr). This amounts to neglecting any contribution from anaerobic glycogenolysis—automatically included in the present approach—and it is bound to underestimate the maximum anaerobic work. Let the initial cytosolic concentrations of ATP and PCr be 10 and 40 mM, respectively, which are quite reasonable values. By taking $\Delta_r G = 50$ kJ/mol, the maximum amount of free energy that the cytosol can liberate through reaction (6.1) is then calculated to be $\Delta G_{\text{Max}} = 50 \times 10^3$ J/mol $\times (10 + 40) \times 10^{-3}$ mol/kg $= 2500$ J/kg per kilogram of cytosolic water. If efficiency is 0.4, the maximum anaerobic work would be 1000 J/kg per kilogram of cytosolic water, which would mean $w_{\max} = 0.68 \times 1000 = 680$ J/kg per kg of wet muscle tissue.

One could also estimate the anaerobic energy capacity of human muscle in the following way (accepting the inherent uncertainties of such determination). We know from experiments that the power output per kilogram of body mass during a maximal 30-s effort on a cycle ergometer averages to about 8 W/kg or a total 240 J/kg [24]. For a 80-kg man this means a total of 19.2 kJ. Assuming a weight 16 kg for the muscles producing this work, the maximum anaerobic work per kilogram of muscle tissue turns out to be given by $w_{\max} = 19.2\,\text{kJ}/16\,\text{kg} = 1200$ J/kg. In this case, the excess of work over the prediction of the present theory ($w_{\max} = 959$ J/kg) is likely to be due to the aerobic energy production taking place after the first 10–15 s of the experiment.

APPENDIX A: ADMISSIBLE RANGE AND ADMISSIBLE SURFACE

A finite system can only store a finite amount of internal energy. This is a consequence of the first law of thermodynamics, stating that in any process

$$\Delta u = \Delta w + \Delta q, \tag{A1}$$

where u, w, and q, in this order, are the specific values (per unit volume) of internal energy, work done *on* the system, and heat absorbed *by* the system. A usual, the operator Δ indicates difference between final and initial values of the variable to which it is attached. In particular,

$$\Delta u = u_1 - u_o, \tag{A2}$$

where u_o and u_1 are the initial and the final values of u, respectively. If the specific internal energy of the system was infinite, no finite input/output of energy (i.e., no amount of $\Delta w + \Delta q$) could change the value of u. This would mean that $\Delta u = 0$ while $\Delta w + \Delta q \neq 0$, in contradiction with the first law (A1). Thus, u must be finite, and such must be Δu as evident from Eq. (A2).

On the other hand, the 2nd law of thermodynamics requires that a finite system can only absorb a finite amount of heat in a process at a finite temperature. This limitation is expressed by the so-called entropy inequality:

$$dq \leqslant T ds, \tag{A3}$$

which refers to any infinitesimal time interval of any process. In this relation, T is the absolute temperature of the system, while s is the system's specific entropy (per unit volume)—a finite-valued function of the state variables of the system. Entropy characterizes the system's capacity of absorbing heat and, therefore, its expression depends on the system. By integrating relation (A3) along a finite time interval of any isothermal process we obtain

$$\Delta q \leqslant T \Delta s. \tag{A4}$$

That is, the amount of heat that a system can absorb at a constant temperature cannot exceed the upper limit $T\Delta s$.

A process is called *loading*, or *unloading*, or *neutral*, if throughout the process the system absorbs work ($\Delta w > 0$), or releases work ($\Delta w < 0$), or does not exchange work at all ($\Delta w = 0$), respectively. From Eq. (A1) we infer that the following relation applies to every unloading process:

$$\Delta u < \Delta q, \tag{A5}$$

since in such a process $\Delta w < 0$. If $\psi = u - Ts$ denotes the system's specific Helmholtz free energy (per unit volume), we have that the relation

$$\Delta\psi = \Delta u - T\Delta s < 0 \tag{A6}$$

applies to every isothermal unloading process, as a consequence of relations (A5) and (A4). In particular, by applying relation (A6) to an infinitesimal interval, we infer that the relation

$$d\psi = d(u - Ts) < 0 \tag{A7}$$

holds true at any time of every isothermal unloading process. Finally, by integrating inequality (A7) along any isothermal unloading process we obtain

$$u - Ts < u_o - Ts_o, \tag{A8}$$

where u_o and s_o denote the initial values of internal energy and entropy, while u and s are the final values of the same quantities.

Because at finite temperature u and s are finite, there will always be one or more states at which ψ attains its largest value, $\psi_{\max} = \max(u - Ts)$, and one or more states at which ψ reaches its smallest value, $\psi_{\min} = \min(u - Ts)$. For a given temperature, the quantities $\psi_{\max}$ and $\psi_{\min}$ represent a fundamental, though seldom acknowledged, constitutive property of the system. From relation (A8) we infer that the following limitation applies to all the states of the system that can belong to an isothermal unloading process at temperature T:

$$\psi = u - Ts \leqslant \psi_{\max}. \tag{A9}$$

The equality sign in the last inequality applies to states where the system's free energy reaches its largest value. Observe that internal energy depends on an arbitrary additive constant or, equivalently, on the reference value assumed for u. However, the same additive constant also enters the definition of ψ and, thus, the value of $\psi_{\max}$. For this reason, relation (A9) holds true irrespective of the choice of the reference value of internal energy.

Let us assume, for simplicity, that at a constant temperature the system enjoys the following two properties:

(i) The system has only one single state in which $\psi = \psi_{\min}$ (*exhaustion state*).

(ii) Starting from any state in which $\psi > \psi_{\min}$, there is at least one isothermal unloading process that brings the system to the exhaustion state.

These properties apply to the vast majority of natural systems, including solutions. Let $\{\boldsymbol{\xi}, T\}$ be the set of the state variables of the system that enter the constitutive equations for internal energy, entropy and, thus, Helmholtz free energy. This means $u = u(\boldsymbol{\xi},T)$, $s = s(\boldsymbol{\xi},T)$, and $\psi = \psi(\boldsymbol{\xi},T)$. (The quantity $\boldsymbol{\xi}$ is an abridged notation to denote the set of all the state variables of the system besides T. For instance, if the system is a solution, $\boldsymbol{\xi}$ will include—but need not be limited to—concentrations of solutes, solute amounts, volume of solvent, etc.) Relation (A9) implies that, at a given temperature T, all the admissible states of a system that meets properties (i) and (ii) above must satisfy the following relation:

$$\psi(\boldsymbol{\xi},T) \leqslant \psi_{\max}. \tag{A10}$$

This relation defines the *admissible range* of the system at temperature T in space of variables $\boldsymbol{\xi}$. The boundary to this range is the system's *limit surface*. It is given by

$$\psi(\boldsymbol{\xi},T) = \psi_{\max}. \tag{A11}$$

Thus, the limit surface is equipotential for ψ.

Function $\psi = \psi(\boldsymbol{\xi},T)$ is determined by the constitutive equations for internal energy and entropy. Therefore, if a state (or point) on the limit surface is known, the value of $\psi_{\max}$ can be determined from Eq. (A11). Beyond the limit surface the system cannot respond according to the original constitutive equations, because this would make it store more energy than it actually can. Thus, the system's response must change as the limit surface is crossed. For instance, the system may break or yield plastically if it is a solid, or it may start to produce precipitates if it is a solution. The appearance of a deviation from the original response is an indication that the limit surface has been crossed. It can be exploited to determine the state of the system at the crossing point of the limit surface.

Any point on the limit surface determines $\psi_{\max}$ from Eq. (A11) and thus the whole admissible range and the whole limit surface from relations (A10) and (A11). This provides complete information on the whole set of states that can be covered by a system of given constitutive equations for internal energy and entropy. We are assuming, of course, that the system is free of external constraints that prevent it from reaching the states on the limit surface.

APPENDIX B: BOUNDED PART OF THE FREE ENERGY OF A SOLUTION

The free energy of a solution is the sum of the free energy of its components. Thus, denoting by $n_{\mathrm{H_2O}}$ and $n_j (j = 1,2,\ldots s)$ the moles of solvent and solutes, the Gibbs free energy of any aqueous solution can be expressed in the classical form:

$$G = n_{\mathrm{H_2O}}\mu_{\mathrm{H_2O}} + \sum_{j=1}^{s} n_j\mu_j + C. \tag{B1}$$

Here μ_j and $\mu_{\mathrm{H_2O}}$ are the *chemical potentials* or partial molar Gibbs free energies of solutes and solvent, respectively, while C is an arbitrary constant.

The chemical potentials can be expressed in the form [6]

$$\mu_j = \mu_j^o(p^o,T) + \bar{V}_j\Delta p + RT\ln a_j \tag{B2}$$

and

$$\mu_{\mathrm{H_2O}} = \mu_{\mathrm{H_2O}}^o(p^o,T) + \bar{V}_{\mathrm{H_2O}}\Delta p + RT\ln a_{\mathrm{H_2O}}, \tag{B3}$$

where a_j and $a_{\mathrm{H_2O}}$ are the effective concentrations (or *activities*) of solutes and solvent, while μ_j^o, $\mu_{\mathrm{H_2O}}^o$, p°, Δp, and R are defined in Sec. II. The quantities $\bar{V}_j$ and $\bar{V}_{\mathrm{H_2O}}$ appearing in the above equations are the *partial molar volumes* of solutes and solvent, respectively. In these equations it is assumed that $\bar{V}_j$ and $\bar{V}_{\mathrm{H_2O}}$ do not depend on pressure, which is true to a good approximation in solids and liquids. Of course, the same equations also apply when $\bar{V}_j$ and $\bar{V}_{\mathrm{H_2O}}$ are pressure dependent, if Δp is appropriately small.

By measuring solute concentrations in molalities (moles of solute per kg of solvent) the quantities a_j can be expressed as

$$a_j = \frac{\gamma_j n_j}{m_{\mathrm{H_2O}}} = \frac{\gamma_j n_j}{n_{\mathrm{H_2O}}M_{\mathrm{H_2O}}}, \tag{B4}$$

the notation being the same as that adopted in Sec. II. On the other hand, by measuring solvent concentration in moles of solvent over total moles of solution components, we have that the activity $a_{\mathrm{H_2O}}$ is given by

$$a_{\mathrm{H_2O}} = \frac{\gamma_{\mathrm{H_2O}}n_{\mathrm{H_2O}}}{n_{\mathrm{H_2O}} + \sum_{j=1}^{s} n_j}. \tag{B5}$$

Because the solution's volume can be expressed as

$$V = n_{\mathrm{H_2O}}\bar{V}_{\mathrm{H_2O}} + \sum_{j=1}^{s} n_j\bar{V}_j, \tag{B6}$$

by introducing Eqs. (B2)–(B6) into Eq. (B1), the Gibbs free energy of an aqueous solution can readily be expressed in the form (2.1). From that equation, the solution's Helmholtz free energy (2.3) is obtained, as discussed in Sec. II. We observe that Eq. (2.3) can be decomposed as

$$\Psi = \Psi' + \Psi'', \tag{B7}$$

where

$$\Psi' = n_{\mathrm{H_2O}}\mu_{\mathrm{H_2O}}^o(p^o,T) + \sum_{j=1}^{s} n_j\mu_j^o(p^o,T) + C, \tag{B8}$$

while Ψ'' is given by Eq. (2.5).

For isothermal processes, the quantities μ_j^o and $\mu_{\mathrm{H_2O}}^o$ are constant and finite, since they are the free energy of a finite amount (one mole) of material in a standard state of pressure and concentration at a finite temperature T. Thus the terms $n_{\mathrm{H_2O}}\mu_{\mathrm{H_2O}}^o$ and $n_j\mu_j^o$ entering Eq. (B8) are the contributions to the solution's free energy coming from the mere presence of the solution components, irrespective of how and whether the said components are mixed to each other, dissolved, or simply put together without even contacting with each other. Because the amount of every component of any system can be chosen arbitrarily, part Ψ' of Ψ is not bounded by thermodynamics. In a finite system, Ψ' is bounded simply because in a finite system $n_{\mathrm{H_2O}}$ and n_j are finite (which is clearly not a thermodynamic restriction!). On the contrary, as apparent from Eq. (2.5), which for better convenience we rewrite here as

$$\Psi'' = RT\sum_{j=1}^{s} n_j \ln\frac{\gamma_j n_j}{n_{\mathrm{H_2O}}M_{\mathrm{H_2O}}} - p^o V. \tag{B9}$$

Ψ'' diverges as $n_{\mathrm{H_2O}} \to 0$ for n_j finite and different than zero. Therefore, because thermodynamics requires that the free energy of any finite system should be finite, Ψ'' must be bounded from above. That is

$$\Psi'' \leqslant \Psi''_{\max}. \tag{B10}$$

By referring this relation to one mole of solvent, inequality (2.8) is finally obtained. For a general treatment of the topic of this section, the reader is referred to [6].

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