

Review Article

An Outlook on Biothermodynamics: Needs, Problems, and New Developments. I. Stability and Hydration of Proteins

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Abstract

The application of concepts, principles, and methods of thermodynamics of equilibria and processes to bioengineering systems has led to a new and growing field: engineering biothermodynamics. This article, which is meant as the first in a series, gives an outline of basic aspects, changes, and actual examples in this field. After a few introductory remarks, the basic concepts and laws of thermodynamics extended to systems with internal variables, which serve as models for biofluids and other biosystems, are given. The method of thermodynamics is then applied to the problem of thermal stability of aqueous protein solutions, especially to that of myoglobin solutions. After this, the phenomenon of hydration of proteins by adsorption and intrusion of water molecules is considered. Several other phenomena like the adsorption of proteins on solid surfaces or cell membranes and their temperature and pressure-related behavior represented by an equation of state, or the thermodynamics of bacterial solutions including chemical reactions like wine fermentation, etc., will be presented in Parts II and III of this article.¹

¹*Labor improbus omnia vincit* (Hard and persistent work will overcome all difficulties).

A. Introduction

The purpose of this article is to introduce the reader to a fairly new field which, despite its old roots, is becoming more and more important these days: biothermodynamics. By this we simply mean – in a first approach – new applications of both classical equilibrium thermodynamics or thermostatics and thermodynamics of irreversible processes to problems emerging in biotechnology and related fields of biosciences and bioengineering.

While most of what will be discussed in this article is well known to thermodynamicists, we sincerely hope to introduce some new aspects of biothermodynamics to those who do not use this subject in their daily work, but feel a need to do so in view of the biotechnical problems they are confronted with.

The very basis of this optimistic view is the fact that, despite the complexity of biological structures like biochemical molecules, proteins, bacteria etc., many phenomena occurring in themselves or in systems including these structures are collective phenomena, i.e., they result from the cooperation of not a few but many identical elements. Also, the universal validity of the laws of thermodynamics and balance equations supports this view strongly. However, we have to admit that the usefulness of thermodynamic descriptions of equilibrium or transient states and of processes in systems of interest to bioengineers can only be proved by presenting the respective results referring to real systems. This sometimes is hampered by the lack of biophysical and thermochemical data, but sometimes also for principle reasons. Despite the introductory and tutorial character of this article, it is also hoped that the examples presented and discussed to some detail in the subsequent sections will help in this respect. Hence, it is also the purpose of this essay to foster discussion and communication between engineers and scientists dealing with the various aspects of biosystems on microscopic (i.e., molecular), mesoscopic, or macroscopic (i.e., industrial) levels. Needless to say, there is an urgent need for research in this field, ranging from basic questions regarding concepts of thermodynamics, such as “state,” “component,” “phase,” etc., to questions of most practical importance; for example, separation of proteins coming from downstream processing or aggregation of proteins as occurring in Alzheimer’s disease.

To be more specific, we would first like to mention the various fields of biotechnology, all of which are growing rapidly today in both scientific and economic importance. Generally speaking, biotechnology refers to all technologies using living systems (single cells, bacteria, fungi, etc.) as chemical reactors to produce or annihilate biochemical molecules, proteins, enzymes, etc., or to support growth processes of other bacterial populations, etc. [A1–A3]. For the sake of simplicity, colors have been assigned to the various fields of biotechnology as follows:

- White biotechnology: Industrial-sized biocatalytic processes, such as fermentation in breweries, production of vitamin B12 in *Pseudomonas denitrificans*, production of steroid hormones, etc. [A4, A5];
- Green biotechnology: Use of plants and their transgene variations for production of biofuels, etc., in biorefineries [A6];
- Red biotechnology: Medical applications of substances and processes related to living organisms, as for example streptomycin, insulin, interferones, etc., to fight bacterial infections, cancer, and other diseases [A7];
- Yellow biotechnology: Design and production of pharmaceutical molecules like recombinant proteins, etc. [A8];
- Blue biotechnology: Seawater-based micro-organisms as chemical reactors, extremophiles (high/low temperatures and/or pressures), nonphototrophic organisms like deep sea bacteria, etc. [A9].

Today, the most important and promising fields of application of thermodynamics are:

- Thermophysical properties like density, compressibility, osmotic coefficients, heat capacity, etc., of biochemical molecules in pure condensed state or in an aqueous or nonaqueous, gel-like solution [A10, A11];
- Phase equilibria of biofluids, i.e., solutions of biomolecules in a solvent fluid (liquid–liquid, liquid–vapor, liquid–solid, osmotic equilibria, etc.) [A12, A13];
- Biocatalysis; especially how the structure and catalytic properties of proteins depend on the pressure, temperature, pH value, and type of solvent of their fluid solution [A14];
- Metabolism of living cells, i.e., the network of chemical reactions within a cell (catabolism and anabolism) and its optimization for growth, life time, substrate acceptance, and production of (one or more) target components [A15];
- Biological or energetic efficiency of bioengineering processes, need for cooling or heating of bioreactors, development of biological refrigerators [A16], biofuel cells [A17], etc.

To apply thermodynamic concepts and methods to any of these fields, it is of utmost importance to define as clearly as possible

- the system one is going to consider and its environment;
- the external exchange processes, i.e., heat and mass transfer, and the internal processes in the system; and

- the level of observation or description at which one would like to describe the system, i.e., the set of variables to be used to describe states of and processes within the system. This requirement also should be observed for any kind of “reference state” being used for the analysis of the system [A18–A20].

In order to get results of practical importance from thermodynamic formalisms and descriptions of any kind of system, it often is necessary to introduce approximations and to make assumptions “from practice,” which obviously do not hold exactly. On principle, there is nothing wrong with this as long as results are handled with care and these approximations are (a) mentioned and (b) taken into account in interpretations.

For the sake of comprehensibility, let us now introduce a simple scheme which allows us to classify the various systems and fields of biothermodynamics already mentioned. This is provided by the size of the system itself or by the size of its characteristic subsystems:

1. Molecular systems

Biofluids, i.e., solutions of big biochemical molecules like proteins, enzymes, etc., in water, alcohol, or other solvents refer to this type of system. They basically can be described by a biochemical extension of “molecular thermodynamics” in the sense of J. M. Prausnitz [A21]. As an example, let us mention highly diluted protein–water solutions. The interactions between the protein and the surrounding water molecules not only lead to a network or even a monomolecular layer of water molecules being quasi adsorbed on the surface and within the protein, but also to long-distance effects. As could be observed recently by using THz spectroscopy, the motion of water molecules is influenced by the protein over a distance of about 1000 molecules. The motion of the far distant water molecules may be compared to that of disco dancers – single and chaotic – whereas the nearer water molecules show some coordination and collective structures in their motion like baroque minuet dancers [A22].

2. Cellular systems

On this level, solutions and solid systems including living cells or bacteria can be described by a thermodynamic formalism in a lumped or coarse-grained manner only. The basic reason for this is that even “simple” bacteria like *Escherichia coli* include typically 5,000–10,000 different chemical components, not all of which are present at one time. Also, the bacterium normally is far away from any kind of equilibrium state and not in a stationary state but rather, due to growth and/or decay processes, in a periodically changing state (limiting cycle) whose parameters (amplitude, frequency)

may change in time. Still, in a broth including many bacteria at one time, collective phenomena will occur which, combined with a highly lumped model of the bacteria's metabolism, may be open to non-equilibrium thermodynamic description; cf. [A23] and the subsequent parts of this article.

3. Technical systems

The prototype of a biotechnical system is the continuously stirred tank reactor (CSTR), ranging in volume from few (μl) to many (m^3) and including living systems like bacteria [A24]. These reactors normally are used for biotechnological purposes, such as brewing of beer, fermentation of wine, etc. However, they also may find medical applications; for example, the identification of bacteria or viruses by means of their heat production during growth processes in substrate, including solutions for medical diagnostics [A25].

The herewith proposed series of articles on various aspects of biothermodynamics is organized according to this scheme of biothermodynamic systems. That is, after a few historic remarks, we first present in this article an outline of thermodynamics and thermodynamics of irreversible processes, emphasizing a fairly old but still not so well known and used concept, namely that of internal variables of a thermodynamic system. These allow, in principle, a more and more refined phenomenological description of the system. They have successfully been applied to describe both states and processes in complex systems, such as liquid crystals [A26] or biological membranes showing active transport [A27].

A very important class of biological molecules is that of proteins. They may be called the building blocks or bricks of life [A30]. They often occur in aqueous solutions where their biological properties strongly depend on their state, i.e., their folding properties or tertiary order. As this order is highly sensitive to changes in temperature (and pressure), it is of practical interest to know the temperature interval in which a protein will remain thermally stable. Thermodynamics can answer this question if certain calorimetric data have been measured and actually are at hand [A31, A32].

This will be demonstrated in Section C of this article.

Proteins in aqueous solutions exhibit quite complex interactions with the water molecules in their surroundings. Actually, water may adsorb to a certain extent on the surface of a protein or intrude in the protein itself (cavern water) and, in so doing, change its structure. A simple thermodynamic formalism which allows us to describe both equilibrium states and relaxation processes of the protein–water system is outlined in Section D (“Hydration of proteins”); cf. the review provided in [A33].

References are given separately at the end of each section of the article.

Proteins may adsorb on nearly any solid or liquid surface – normally except themselves. This is due to their heterogeneous surface structure, including polar and nonpolar atomic groups. This phenomenon, namely “adsorption of proteins” will be discussed in Part II of this article series. Part II will also give a thermodynamic description of states and processes within a protein after adsorption on a surface and discuss this to a certain extent. An interesting example for protein adsorption with considerable technical potential is provided by the common barnacle. This is known to produce a certain protein that serves as a glue which allows the creature to fix itself to surfaces of all kind. A special feature of this protein-glue is its resistance to water, whereas most glues used today are subject to water corrosion, leading finally to a breakdown of the adhesive properties of the glue.

In Part III of this series, we will discuss a rather classical application of thermodynamics in biological systems; namely, the photosynthesis process of plants.

It will be shown that plants (and other phototrophs) use, besides infrared radiation, the evaporation of water to get rid of entropy, which is necessary to form low-entropy products like sugar/glucose from high-entropy products like carbon dioxide and water at nearly thermal equilibrium with their surroundings [A28]. In view of the global warming problem posed by carbon dioxide (and other greenhouse gases), this is extremely important because a worldwide reforestation program would provide a buffer or even a natural storage system of utmost importance for these gases.

In this part, we will also address the question of whether the behavior of biological structures at varying pressure/temperature conditions can be described by thermodynamic equations of state. As will be shown, this is actually possible.

Let us consider a biological membrane that shows phase transitions at certain pressures and temperatures which change their transport properties, i.e., permittivity for other molecules. An equation of state (EOS) is developed from the available experimental data, which provides information on the state of the membrane at arbitrary pressures and temperatures [A29].

Thermodynamic aspects of the metabolism of bacteria and of bioreactors will be discussed in Part IV of this series of articles.

Section E (“Concluding remarks”) of this article emphasizes some general aspects of thermodynamics and thermodynamics of irreversible processes when applied to biological systems and also mentions several phenomena in

biofluids and proteins that have not been discussed here as it would exceed our printing space and time.

Another field of biotechnology that really deserves a thorough thermodynamic description is the so-called downstream processing of biological broths leaving a reactor, i.e., all the separation processes necessary to isolate one or more wanted biomolecules from myriads of other components. Actually, there are several articles and conference proceedings available dealing with this field [A34, A35]. These serve very well as starting points for further analysis and research.

Summarizing the analysis given so far, it can be stated that biofluids, i.e., mixtures of big biochemical molecules and small molecule solvents, cannot be adequately described today by traditional thermodynamic variables, i.e., temperature, pressure, and amounts of all components. A similar statement holds for the behavior of biochemical molecules like proteins, enzymes, etc., themselves, i.e., interaction phenomena with the solvent molecules leading to defolding, refolding, etc. We herewith propose to extend the traditional thermodynamic description by introducing new, so-called internal variables and provide the thermodynamic formalisms which would allow us to describe both equilibria states and processes in the respective systems. If possible, these new variables should always be given a physical interpretation. This basically would facilitate the use of approximations and also would make it possible to evaluate and interpret results.

Whenever possible, results of “in silico experiments,” i.e., numerical simulation, should be taken into account, although evaluation of these sometimes may be difficult. However, as far as interactions between biomolecules are concerned, some progress has been achieved and the respective software tools are commercially available [A36]. A similar statement holds with regard to experimental methods now available to analyze and characterize biofluids and even single biomolecules [A37, A38].

One of the basic advantages of the method of thermostatics and thermodynamics of irreversible processes is that, no matter how small or even scarce the available information of a system is, the description always can be complemented by a thermodynamic optimization procedure including, for equilibrium situations, maximization of entropy at constant internal energy, etc., and, in the case of processes, the minimization or maximization of the entropy production. These procedures also may be interpreted as “unprejudiced guesses”; the results, however, are often of very limited importance.

Nevertheless, it should also be emphasized that in trying to apply thermodynamic concepts and methods to biological systems, several problems arise

which still are open and whose solution probably requires the redefining and extension of some of the basic concepts of thermodynamics, such as “component,” “phase,” etc. To be specific:

- The “system with subsystems problem”: Thermodynamic systems with a varying number of subsystems can be described in different ways. A fermenter with a growing population of bacteria, each of which is again considered to be a system of its own, is an example for this type of systems [A39, A40];
- The “big molecule problem”: Big molecules like proteins, enzymes, etc., interacting with many small molecules of a solvent, which in turn may adsorb or even penetrate the big molecule. Today it is not quite clear which and how many new variables have to be introduced to achieve a phenomenological description useful for even the biochemical engineer. What will be presented in the subsequent sections of this article can only be considered as a first step and indeed is of limited applicability and importance.
- The “unused information problem”: This is the fact that there is a lot of information on biomolecules available today both on paper and in the Web. However, it often is not acknowledged by scientists from other fields of science and engineering, although it could be very useful to them. For example, all the “-omics” sciences, whose many and sometimes really useful findings do not normally come to the attention of the bioengineer, who often would be grateful to have them at hand in an appropriate manner. This finally leads us to
- The “communication problem”: Molecular biologists, biochemists, biophysicists, biomathematicians, and bioengineers are using quite different languages in science. Also, words used in common often have quite different meanings and semantics. Here one can only hope that by organizing conferences emphasizing research, as for example the Gordon Research Conference (GRC) in the United States and abroad, interdisciplinary research and communication will be promoted to the benefit of all those participating and to society as a whole. There is still truth in the (ancient) proverb: *Si volo pacem et prosperae, para colloquium*. (If you want peace and prosperity, prepare a conference.)

Many more problems could be mentioned that have the potential to retard the future development of biothermodynamics. Nevertheless, we hope to convince the reader with this article and with all the articles and books cited herein that this is a fascinating field, one which deserves more research activity and interdisciplinary exchange of knowledge at all levels of description related to molecular, cellular, and macroscopic biotechnical systems.

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B. A note on thermostatics and thermodynamics of processes

In this section we will give a short overview of the basic structure and statements of classical thermostatics and thermodynamics of irreversible processes. We will restrict ourselves to fluid single-component systems with internal variables which serve as models for various types of biochemical and biological systems such as aqueous solutions of proteins, bacteria, etc. As the literature of classical thermodynamics is abundant, we will have to restrict ourselves here to only a few books, which will be cited for readers interested in more details [B1–B10].

Thermodynamics is

- a phenomenological system theory, i.e., a method to describe equilibria states and processes in and interactions between thermodynamic systems and their environment, especially
- transformations of mass and energy which themselves depend on the
- thermophysical properties of the system considered, i.e., the reaction of the system to changes in pressure, temperature, pH data, etc., in its environment.

Basic to every type of thermodynamic formalism is the concept of a thermodynamic system, coined as late as 1929 by W. Schottky [B5, B11]:

“In nature there are systems, i.e. sets of bodies, separated from their surroundings by clearly defined boundaries and interacting with it only by the exchange of heat, work and mass [cf. Figure 1]. Such systems can be called thermodynamic systems (Σ).” (Author’s translation.)

The thermodynamic system sketched in Figure 1 has in any state a well-defined volume (V), an internal energy (U), and a certain mass (m). To describe a state of the system phenomenologically in more detail, we assume that the system also has a so-called “internal variable (ξ)” which is of extensive character. The nature of this variable will be outlined in more detail afterwards. There also examples will be given.

From the extensive quantities mentioned so far, so-called accompanying intensive quantities, namely a temperature (T), pressure (p), chemical potential (μ), and an “affinity” (A) related to (ξ) can be defined via the fundamental

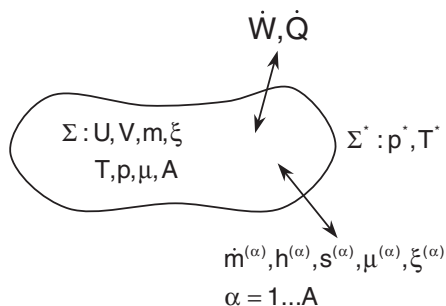


Figure 1 Sketch of a simple thermodynamic system (Σ) and its environmental system (Σ^*). Exchange quantities between Σ and Σ^* are: mechanical work (\dot{W}), heat (\dot{Q}), and mass flows $\dot{m}^{(\alpha)}$ or molar flows $\dot{n}^{(\alpha)}$, $\alpha = 1 \dots A$, the index (α) indicating the number or location of the aperture in the boundary surface of the system. No surface phenomena, external forces, or radiation phenomena are taken into account.

entropy relation, $S = S(U, V, m, \xi)$, and the Gibbs equation [B1–B10]. This will be outlined in the subsequent sections.

The system (Σ) also is assumed to be an open system, i.e., to have a certain number (A) of apertures ($\alpha = 1 \dots A$) at which mass in state ($Z^{(\alpha)}$) with flow ($\dot{m}^{(\alpha)}$), specific enthalpy ($h^{(\alpha)}$), entropy ($s^{(\alpha)}$), chemical potential ($\mu^{(\alpha)}$), and internal variable ($p^{(\alpha)}$) may enter or leave the system.

The surroundings or environment of a thermodynamic system (Σ) may also be considered to be a thermodynamic system (Σ^*). Here we assume that its temperature (T^*) and pressure (p^*) are constant. Relations describing exchange processes of heat, mechanical or electrical work, and mass between (Σ) and (Σ^*) should be invariant against a change of system and environment ($\Sigma \leftrightarrow \Sigma^*$). This requirement may be called the principle of environs invariance [B12]. It is a generalization of the so-called principle of dynamic symmetry long known in mechanics and electrodynamics [B13] and has proved to be useful in developing nonlinear rate equations for heat and mass transfer [B14].

The definition of a thermodynamic system given above can be generalized in various ways. We mention here only those which might be of importance to biological and bioengineering systems:

- the **boundaries** of the system do not necessarily need to be smooth, i.e., continuous and differentiable curves or surfaces, but may have a fractal or even fuzzy structure, leading to the concepts of
- a thermodynamic system or phase of fractal dimension as for example aggregates of adsorbed organic molecules or enzymes in porous sorbent materials [B15, B52], or

- a fuzzy thermodynamic system, as for example boundary layers of fluids near surfaces or material matter in a critical state;
- **external forces** like electromagnetic fields, the gravity field of the earth, or centrifugal forces sometimes have to be taken into account, as phenomena like dielectric bioimpedances [B16, B17], biomagnetism [B18], and biosedimentation are interesting phenomena of growing technical importance;
- **exchange of information** (signals) between a thermodynamic system and its environment also may be considered; this may lead to a change in a properly defined entropy of the system but only to a negligible change in its internal energy, causing subsequent processes within the system itself and possibly new exchange processes with its environment.

In what follows we will restrict ourselves to so-called **simple** thermodynamic systems. These are defined as thermodynamic systems in which surface phenomena, exchange of radiation energy with its surroundings, and also effects of external forces can be neglected. These systems are considered to be models for biological or living systems, i.e., we restrict ourselves in what follows to those aspects of biological systems which can be described by processes in simple thermodynamic systems as defined above (Figure 1).

The structure of a thermodynamic theory describing equilibria states and processes in a thermodynamic system (Σ) basically depends on the number and type of external operations or exchange processes between the system (Σ) and its environment (Σ^*) considered, and the type of internal processes (i.e., processes occurring within the system itself) that are taken into account. The set of these processes and/or operations defines the so-called “level of observation” (*Beobachtungsebene*) at which the system is described.

External operations such as changes in pressure, temperature, pH value in the vicinity of a system or the exchange of mass lead, as far as changes in the equilibria states of a system are concerned, to thermostatic equations of state (EOS), for example:

- exchange of mechanical work: thermal EOS;
- exchange of heat: caloric EOS;
- exchange of mass: chemical EOS;
- exchange of electromagnetic energy: dielectric EOS and magnetic EOS [B16–B18].

Similarly, kinetic or dynamic relations – so-called constitutive equations – have to be developed for the respective processes. Examples for these will be given later.

The state of a thermodynamic system can phenomenologically be described in more detail by introducing more variables, often called “internal variables” and normally being related to the molecular structure of the system. These variables were originally introduced by P. W. Bridgeman in the 1920s in developing thermodynamics of ferromagnetic materials [B23]; they have since been used extensively by Meixner, Kestin, Muschik and others [B19–B22]. Some examples for systems and processes with internal variables are:

1. System (S): Quartz glass in either amorphous (i.e., transparent) state or crystalline (i.e., blind) state.
 Internal process (IP): Phase change or transformation from amorphous to crystalline state.
 Internal variable (IV): Mass fraction of glass in amorphous state.
2. S: Black iron including carbon atoms located on energetically different intracrystalline sites of type α , β [B2, p. 147].
 IP: Diffusion of carbon atoms from α -sites to β -sites and vice versa caused by external mechanical stress or temperature gradients (Snoek effect).
 IV: Numbers of carbon atoms on α -sites and β -sites per mol of Fe-atoms.
3. S: Single protein in aqueous solution including water adsorbed on its external or internal surface, cf. Section D.
 IP: Adaptation of protein structure to amount of water adsorbed (water induced re- or denaturalization process).
 IV: Number of adsorption sites for water on the external or internal surface of the protein, not all of which necessarily have to be occupied by a water molecule.
4. S: Single protein adsorbed on solid surface.
 IP: Change of protein's structure or folding due to adsorption-based interactions with the surface [B30, B31].
 IV: Number of atomic contacts between protein and solid surface; cf. Part II of the article.
5. S: Closed bioreactor including a bacterial population in substrate solution.
 IP: Metabolism and catabolism of bacteria.
 IV: Extent of metabolic and catabolic reaction (reaction numbers) [B49, B50].

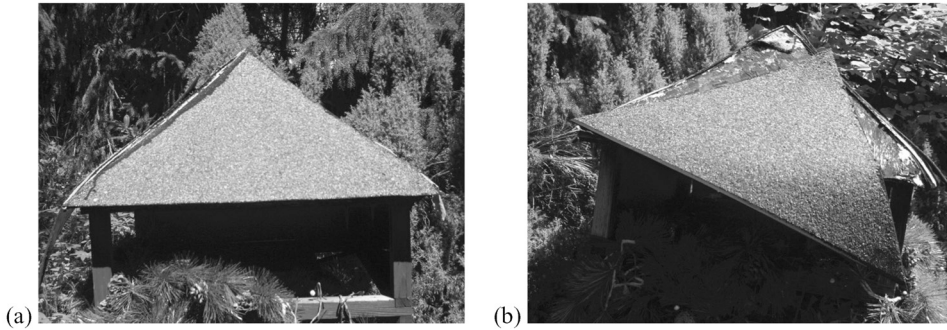


Figure 2 (a) Birdhouse in author's neighbor's garden in its initial state. The paper-based roofing is pasted to the wooden roof underneath. State: restricted or inhibited equilibrium state. (b) Bird house after 10 years. Sliding down of roofing: internal process approaching total or unrestricted equilibrium when the roofing has fallen down to earth. State: external equilibrium, internal non-equilibrium. Internal variable: position of triangular roofing.

As the physics related to “internal variables” of thermodynamic systems often is somewhat sophisticated, it seems to be appropriate to present the reader two examples “from every day life” more explicitly.

Example 1: In one of the author's neighbor's gardens there is a birdhouse for feeding the birds in winter time. The house is protected against rain by paper-based roofing material pasted with glue to the wooden roof underneath (see Figure 2a).

The birdhouse can be considered as a thermodynamic system being in (thermal, etc.) equilibrium with the surrounding air. This state seemed to be maintained for days, weeks, and even months. However, after 10 years or so, it became obvious that the glue used to fix the roofing did not harden properly but remained over the years in a kind of plastic state, which caused the (light-weight) roofing to slide down slowly as the result of gravity. The state resulting after 10 years is shown in Figure 2b. Today, the roofing is still moving slowly downwards and will eventually find its final position on the ground if not prevented from falling by being properly repaired and fixed with nails in its original position (Figure 2a).

Figure 2b proves that the “state” of the birdhouse shown in Figure 2a only refers to “external equilibrium,” but internally the roofing right from the beginning started to slide down slowly to reach the position shown in Figure 2b after 10 years. In engineering, situations like this can be characterized by so-called “Deborah numbers,” actually indicating time scales or durations of operation or observation of a system. Let (x) be any geometric coordinate indicating the position of the triangular roofing on the birdhouse and $\dot{x} = dx(t)/dt$

the velocity of the roofing's motion. Then the Deborah number of this process can be defined as

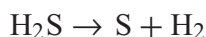
$$De = \left| \frac{\dot{x}}{x} \right| t_{OBS} \quad (B0)$$

Here (t_{OBS}) is the time interval during which the "system" is observed, i.e., changes in its "state" are registered. For short times of observation, i.e., $t_{OBS} \simeq 1$ week, $De \ll 1$ and the sliding process of the roofing may be neglected. However, for long times of observation, i.e., $t_{OBS} \simeq 10$ years, $De \simeq 1$ or $De > 1$ and sliding should be taken into account as an "internal process" approaching a final equilibrium state.

In view of these considerations, the initial state of the birdhouse, Figure 2a, can be called an "external only" or restricted or frozen equilibrium as the roofing is already sliding down, albeit very slowly. Also, the sliding process can be considered an "internal process," which however only has to be taken into account for long times of observation, i.e. $t_{OBS} \simeq 10$ years. When the roofing has finally fallen down after another couple of years, or its motion has been stopped in any other position, we may call this situation a full or unrestricted equilibrium state in which no "internal processes" are occurring any longer.

Example 2: This example refers to an adsorption experiment during which the uptake of hydrogen sulfide (H_2S) gas in a sample of highly porous activated carbon has been measured by weighing the sample using a magnetic suspension balance [B15]. The lower curve shows the relative increase in weight of the sample as a function of time, with the value "1" referring to the maximum uptake, i.e., the equilibrium value. As can be seen, adsorption equilibrium is reached after 3–4 minutes (Figure 3).

The upper curve shows data of the dielectric permittivity of the sample which was placed within an electric capacitor. Again, the equilibrium value of the permittivity has been assigned the value "1." The curve reflects an internal relaxation process that occurs within the gas-loaded sample, though – according to the lower curve – its mass is practically constant. This can be explained by the fact that the carbon acts as a catalyst to the hydrogen sulfide, i.e., in the sample the dissociation reaction



takes place, leaving the mass of the sorbent–sorbate sample constant but changing its dielectric permittivity considerably until, after approximately 1 hour, full or unrestricted equilibrium has been reached.

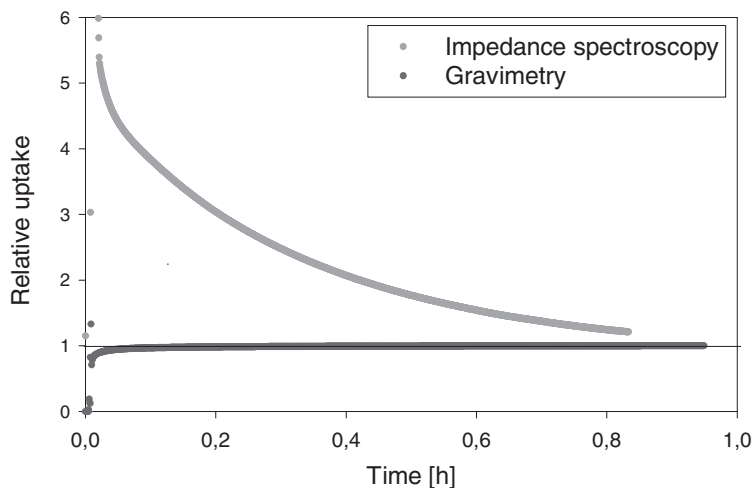


Figure 3 Gravimetric and dielectric measurements of the uptake process of the H_2S gas in a sample of activated carbon. The upper curve refers to the dielectric permittivity, the lower curve to the microbalance signal, i.e., the weight of the sample [B15].

Internal variables often, but not necessarily, are extensive quantities for which balance or rate equations can be formulated, including storage, flow, and production terms [B2, B9]. The internal processes related to them often are governed by time scales much longer than those describing the approach of the system to “external equilibrium” (cf. Figure 3) or to a stationary state, i.e., for example mechanical or thermal equilibrium with its surroundings, or a stationary state with respect to the exchange of work, heat, and mass between the system and its surroundings. Hence, these internal variables can be considered independent variables approaching however equilibrium or stationary (i.e., constant) values which are completely determined by the set of external (intensive) variables of the system’s surroundings [B5, B23].

To elucidate this statement by another example, we mention that proteins being adsorbed from an aqueous solution on an organic membrane at constant temperature and pressure may undergo an unfolding, i.e., a denaturation process, that can last for hours, days, and sometimes weeks. The number of contacts (i.e., atomic groups of the protein interacting with atoms of the membrane or the “surface” of the – partly unfolded – protein, presented by the number of adsorption sites for polar or ionic molecules from the surrounding aqueous solution) is an example for internal variables that allow the description of this situation phenomenologically within a thermodynamic formalism.

Equilibria states in which the internal variables of a system assume arbitrary values, normally different from their equilibrium values corresponding to

given and constant external conditions, are called restricted or inhibited or frozen equilibria states (*gehemmte Gleichgewichte*). These states may still vary slowly in time.

Equilibria states in which the internal parameters do not change but have assumed their equilibrium values are called total, full, or external and internal equilibrium states; cf. Figure 3 and Section D.

Similarly, we will call stationary states of open thermodynamic systems either restricted or total depending on whether the internal parameters of the system have already or have not assumed their stationary state values corresponding to the external intensive parameters and the flows of work, heat, and mass through the system, respectively.

All states of a thermodynamic system that are neither stationary nor equilibrium states will be called non-equilibrium states. These generally will occur if the system is forced by rapid changes in environmental parameters like pressure, temperature, pH value, etc. to transit from its initial equilibrium state to another state, which does not necessarily have to be an equilibrium state again.

More examples for the use and application of the concept of internal variables in biothermodynamic systems will be given in Section D and subsequent Parts II–IV of this article.

The basic laws of thermodynamics

In this section we are going to formulate the laws of thermodynamics for a simple thermodynamic system (Σ) including a single component of mass (m) and mole number (n) and being characterized by an extensive internal variable (ξ); cf. Figure 1.

0th Law of Thermodynamics [B3, B5]

The system Σ has in any equilibrium state an intensive quantity of state: the absolute temperature, T . It is related to the pressure within the system, p , its volume, V , mole number, n , and – for restricted equilibria or non-equilibria states – the internal variable ξ by the thermal equation of state (TEOS) of the system:

$$\phi(p, V, T, n, \xi) = 0. \quad (\text{B1})$$

The function Φ may be converted to the temperature-explicit form

$$T = T(p, V/m, \xi/m), \quad (\text{B2a})$$

or pressure-explicit form

$$p = p(T, V/m, \xi/m) \quad (\text{B2b})$$

as actually needed in process calculations.

For equilibrium states, p and T can be directly measured by standard procedures [B1–B6]. For stationary or non-equilibrium states, p and T are defined as “accompanying equilibrium pressure and temperature” via the caloric equation of state (CEOS), cf. (B3b) and the second law of thermodynamics (Part 1), i.e., the Gibbs fundamental equation (B6), (B8) [B2–B4].

1st Law of Thermodynamics

The system Σ has in any state an extensive quantity: the internal energy, U . For equilibria states it is related to the volume, V , the temperature, T , the mass, m , or mole number, n , and – in case of restricted or frozen equilibria – to the internal variable ξ of the system by the caloric equation of state (CEOS) as

$$\Psi(U, V, T, m, \xi) = 0, \quad (\text{B3})$$

which also may be written in the energy-explicit form

$$U = U(T, V, m, \xi) \quad (\text{B3a})$$

or in the enthalpy form [B3, B26–B28]:

$$H = U + pV = H(p, T, m, \xi). \quad (\text{B3b})$$

The internal energy of the system Σ (Figure 1) can be changed by the exchange of heat Q , volume-related work ($dW = -pdV$), and mass flows $\dot{m}^{(\alpha)}$.² Hence, the energy balance is

$$dU = dQ - pdV + \sum_{\alpha=1}^A h^{(\alpha)} dm^{(\alpha)}. \quad (\text{B4})$$

Here $h^{(\alpha)}$ is the molar enthalpy of the mass flow $\dot{m}^{(\alpha)}$ at position ($\alpha = 1 \dots A$).

For isolated systems, all energy flows on the r. h. s. of Eq. (B4) vanish and we get

$$dU = 0, V = \text{const}, \quad (\text{B5})$$

²For the sake of simplicity, we refrain from considering a general (technical) work term (dW_t) as it would imply the introduction of more internal variables and the concept of a non-equilibrium entropy in the formulation of the Second Law. However, we will present these considerations in a subsequent part of the article.

which is the famous law of conservation of energy [B2, B3, B53]. Physically it is rooted in the invariance against time translation transformations of the underlying equations of motions of the atoms and molecules of the system as was shown by E. Noether in the 1930s [B53].

2nd Law of Thermodynamics

The thermodynamic system Σ , Figure 1, has in every equilibrium state an extensive quantity: the entropy [B1–B3, B29, B39]:

$$S = S(U, V, m, \xi). \quad (\text{B6})$$

As the extensive variables ($U \dots \xi$) are also well defined in transient and non-equilibrium states, one formally can also assign the entropy as “process accompanying thermostatic entropy” to these states [B2, B38, B41, B43].

According to Max Planck, S is a phenomenological measure for the tendency, preference, or thermodynamic probability of a system to actually realize the (restricted or unrestricted) equilibrium state characterized by a set of numerical values (U, V, m, ξ). Other interpretations of S have been given by L. Boltzmann (1890) [B7, B32] and C. Shannon (1945) [B32–B34]. According to Ludwig Boltzmann, S is a logarithmic count of the “microstates of the system,” i.e., the number of possibilities of how atoms and molecules of a system can be arranged in a well-defined phase space at given external parameters (U, V, m, ξ); cf. Eq. (B6). Although this interpretation has proved to be very useful for ideal gases, liquids, and crystals, one should not overlook its limitations for situations where the set of microstates becomes uncountable, as for example in the folding problem of chains of amino acids in three-dimensional space. leading to native protein molecules.

Genuine extensions of the concept of entropy have been given in modern theories of irreversible processes in continuous matter, such as extended thermodynamics, mesoscopic thermodynamics, and finite time thermodynamics. An overview of these is given in [B5]; cf. also [B19–B25, B35–B38].

The second part of the Second Law of Thermodynamics states that in the system (Σ) only transfer processes between equilibrium states ($Z_0 \rightarrow Z$) can be realized for which the inequality holds [B1, B30, B38, B51]:

$$S(Z) - S(Z_0) \geq \int_{Z_0}^Z \left(\frac{dQ}{T^*} + \sum_{\alpha=1}^A s^{(\alpha)} dm^{(\alpha)} + \frac{A}{T} d\xi \right). \quad (\text{B7})$$

This is the famous inequality of R. Clausius, extended here to systems with external mass exchange ($m^{(\alpha)}$) and one internal variables (ξ).

The inequality sign holds for all natural, i.e., irreversible processes. The equality sign holds for quasi-static reversible processes. In Eq. (B7), T^* is the temperature at which either heat is transferred from the environment (Σ^*) to the system (Σ) or withdrawn from the system. Also $s^{(\alpha)}$ indicates the specific entropy of mass flow $\dot{m}^{(\alpha)}$ ($\alpha = 1 \dots A$) to or from the system and A is the so-called affinity of the internal variable ξ of the system. As we shall see later, this phenomenological parameter can be related to the entropy production of the internal process occurring during the transfer of the system from a restricted equilibrium state to a full or unrestricted equilibrium state characterized by the (necessary but not sufficient) condition: $A = 0$.

From a molecular point of view, both parts of the Second Law of Thermodynamics, namely the existence of entropy (B9) and the Clausius inequality (B10), are consequences of the existence of molecules and the law of large numbers and/or the central limit theorem in mathematics. This was shown by L. Boltzmann in the 1890s and was later on extended by van Kampen, Balescu, Meixner, and others [B32].

For quasi-static changes of state, i.e., a sequence of equilibria states ($Z \rightarrow Z + dZ$), the inequality (B7) can be formulated locally in time, i.e.,

$$dS \geq \frac{dQ}{T^*} + \sum_{\alpha} s_m^{(\alpha)} dm^{(\alpha)} + \frac{A}{T} d\xi. \tag{B7a}$$

As one can recognize from both inequalities (B7) and (B7a), the entropy of the system (Σ) can be changed by the exchange of heat and mass and by internal processes. For reversible infinitesimal changes of state, i.e., heat transfer between ($\Sigma \leftrightarrow \Sigma^*$) at vanishing temperature differences ($T = T^*$), exchange of mechanical work at vanishing pressure differences ($p = p^*$), and mass exchange at equilibrium conditions ($h^{(1)} = \dots h^{(A)} = h$, $s^{(1)} = \dots s^{(A)} = s$, $\xi^{(1)} = \dots \xi^{(A)} = \xi$), the Second Law (B7a) with the equality sign (=) can be combined with the First Law (B4) to give the famous Gibbs fundamental equation:

$$dS = \frac{1}{T} dU + \frac{p}{T} dV - (\mu/T) dm + (A/T) d\xi. \tag{B8}$$

Here the chemical potential $\mu = h - Ts$ of the material in the system Σ has been introduced. Also we have used the notation $dn = \sum_{\alpha=1}^A dn^{(\alpha)}$. The Gibbs equation can be rewritten for the free enthalpy $G = U + pV - TS$ of the system as

$$dG = -SdT + Vdp - \mu m + Ad\xi. \tag{B9}$$

If the function $G = G(T, p, m, \xi)$ is known explicitly or is approximated by a Taylor series expansion of (at least) second order, the equations of state (EOS) of the system can be easily calculated according to (B9) as:

$$\left(\frac{\partial G}{\partial T}\right)_{p,n,\xi} = -S(T, p, m, \xi) \quad \dots \text{entropic EOS} \quad (\text{B10})$$

$$\left(\frac{\partial G}{\partial p}\right)_{T,n,\xi} = V(T, p, m, \xi) \quad \dots \text{thermal EOS} \quad (\text{B11})$$

$$\left(\frac{\partial G}{\partial n}\right)_{T,p,\xi} = \mu(T, p, n, \xi), \quad \dots \text{chemical EOS} \quad (\text{B12})$$

$$\left(\frac{\partial G}{\partial \xi}\right)_{T,p,n} = -A(T, p, m, \xi), \quad \dots \text{internal EOS} \quad (\text{B13})$$

The equilibrium principle for the free enthalpy

$$dG = 0, d^2G > 0. \quad (\text{B14})$$

delivers according to Eq. (B9) for

a) external equilibria only, i.e., internal non-equilibria states ($A \neq 0$):

$$T = \text{const}, p = \text{const}, m = \text{const}, \xi = \text{const}; \quad (\text{B14a})$$

b) external and internal or full equilibria states:

$$\begin{aligned} T = \text{const}, p = \text{const}, m = \text{const}, \\ A(p, T, m, \xi) = 0; \end{aligned} \quad (\text{B14b})$$

from which the equilibrium value of the internal variable can be calculated as

$$\xi_{\text{eq}} = \xi_{\text{eq}}(p, T, m). \quad (\text{B15})$$

Thermodynamics of irreversible processes

The thermostatic equations of state (B10)–(B13) only allow to calculate from given parameters of a system (T, p, n, \dots, ξ) the corresponding variables, i.e., equilibrium entropy, volume, chemical potential, and affinity (S, V, μ, A) or vice versa. However, kinetic processes like transfer of thermal energy (heat),

mechanical work, or mass to or from the system cannot be described. In order to achieve this, kinetic or constitutive equations for these processes must be formulated. A sound physical basis for this is provided by the Clausius inequality (B7), which can be rewritten as:

$$\int_{-\infty}^{\infty} \left\{ \dot{S}(t) + \frac{\dot{Q}}{T^*} + \sum_{\alpha=1}^A s^{(\alpha)} \dot{m}^{(\alpha)} + \frac{A}{T} \dot{\xi} \right\} dt > 0. \tag{B16}$$

Here (S) indicates the so-called accompanying thermostatic entropy of the system, i.e., the entropy it would have if it were, after sudden isolation from its surroundings at time (t) in a (full or restricted) equilibrium state.³ Also, we have assumed that the system (Σ) starts at $t = -\infty$ from an equilibrium state (Z_0), undergoes a process, and then approaches for $t \rightarrow \infty$ another equilibrium state (Z). The integrand in Eq. (B16) can be rearranged by using for (\dot{S}) the Gibbs equation (B8) for full accompanying equilibrium states, i.e., $A = 0$, but not for the actual process, and the First Law of Thermodynamics (B4) to get a sum of products of thermodynamic forces and fluxes, i.e., quantities which vanish in equilibrium states and are invariant or not invariant, respectively, against time reversal transformations [B3, B6, B43]. We get from Eq. (B16) the inequality

$$\int_0^t \left\{ \left(\frac{1}{T} - \frac{1}{T^*} \right) \dot{U} + \left(\frac{p}{T} - \frac{p^*}{T^*} \right) \dot{V} + \sum_{\alpha=1}^A \left(\frac{\mu^{(\alpha)}}{T^*} - \frac{\mu}{T} \right) \dot{m}^{(\alpha)} - \frac{A}{T} \dot{\xi} \right\} dt \geq 0 \quad \dots \text{all } t \geq 0 \tag{B17}$$

Here we have assumed the exchange processes between ($\Sigma \leftrightarrow \Sigma^*$) to start at $t = 0$. Also, assuming the system (Σ) to be suddenly isolated from its environment (Σ^*), i.e., ($\dot{U} = 0, \dot{V} = 0, \dot{\gamma}_q = 0, \dot{n}_i = 0 \dots$ all $t' \geq t$) and also to be frozen internally ($\dot{\xi}_k = 0, t' > t$), one recognizes that inequality (B45) must hold for all times $t \geq 0$.

Modified versions of Eq. (B17) were called “Fundamental inequalities of thermodynamics of processes” in 1969 by J. Meixner and others [AH, IK].

³This quantity should not be mixed up with any kind of “non-equilibrium entropy,” as this quantity cannot be defined in an unequivocal and clear-cut way, a fact which clearly has been recognized and pointed out by J. Meixner [B40] and also by the author [B5, B41]. The use of a “non-equilibrium entropy” has proved to be useful, as for example in so-called “extended thermodynamics of irreversible processes,” cf. [B21, B37]. However, the main physical results of this theory also can be derived without the concept of an entropy of a system in non-equilibrium states [B36, B42].

We will follow this tradition in order to honor J. Meixner, who really has contributed to the development of thermodynamics of irreversible processes in various respects [B38, B40, B44, B45, B51].

The integrand in Eq. (B17) is the entropy production related to the processes occurring in the system (Σ). The terms are respectively related to the exchange of thermal energy (heat), of mechanical work, to mass transfer to and from (Σ), and to internal processes.

In exploiting the inequality (B17) we restrict ourselves in view of systems and processes presented in the subsequent sections C and D to isothermal and isobaric processes, i.e.; we assume $T = T^* = \text{const}$, $p = p^* = \text{const}$. Then Eq. (B17) reduces to

$$\int_0^t \left\{ \sum_{\alpha=1}^A (\mu_i^{(\alpha)} - \mu_i) \dot{m}^{(\alpha)} - A \dot{\xi} \right\} dt \geq 0. \quad (\text{B18})$$

This inequality shows the intrinsic coupling between mass transfer to or from Σ and the internal process (A, ξ); for example, denaturation processes of proteins within the system.

Numerical calculations of isothermal–isobaric processes in Σ can be performed as follows: We define the “state” of (Z) of the system Σ at a certain time (t) as given by

$$\Sigma : Z(t) : T = \text{const}, p = \text{const}, m(t), \xi(t). \quad (\text{B19})$$

From these quantities the numerical values of the intensive variables (μ, A) of Σ at time (t) can be calculated via the thermostatic potential function $G = G(t, p, m, \xi)$, i.e., the equations of state (B12) and (B13),

$$\mu(t) = \frac{\partial}{\partial n} G(T, p, m, (t), \xi(t)), \quad (\text{B20})$$

$$A(t) = \frac{\partial}{\partial \xi} G(T, p, m, (t), \xi(t)). \quad (\text{B21})$$

Now, in view of the inequality (B18), the thermodynamic flows of the system,

$$\dot{m}^{(\alpha)}, \dot{\xi}, \quad \alpha = 1 \dots A, \quad (\text{B22})$$

cannot be independent of the “thermodynamic forces,” namely their coefficients in the integrand of Eq. (B18), which basically is the entropy production of the system:

$$\mu^{(\alpha)} - \mu, -A, \quad \alpha = 1 \dots A. \tag{B23}$$

Hence, we assume in the sense of Eckart and Onsager process equations to hold as [B2, B3, B6, B40, B42]

$$\dot{m}^{(\alpha)} = M^{(\alpha)} \quad (. /.), \quad \alpha = 1 \dots, \tag{B24}$$

$$\dot{\xi}^{(\alpha)} = \Xi \quad (. /.), \tag{B25}$$

$$(. /.) = (T = \text{const}, \mu^\beta - \mu, A, \quad \beta = 1 \dots A).$$

The symbols $M^{(\alpha)}, \Xi, \alpha = 1 \dots A$, represent either functions or functionals of their arguments referring to the history of the system ($-\infty < s \leq t$).

These functions or functionals are due to several conditions that have been extensively discussed in the literature and therefore are omitted here [B6, B9, B12, B14, B21, B22, B24, B36, B42, B46, B51].

Using the process equations (B24) and (B25) the state $Z(t + \Delta t)$ of the system Σ at some later time $(t + \Delta t)$ can be calculated as

$$\begin{aligned} \Sigma : \quad Z(t + \Delta t) : \quad T = \text{const}, \quad p = \text{const} \\ m(t + \Delta t) = m(t) + \dot{m}(t)\Delta t + \vartheta \left((\Delta t)^2 \right) \\ = m(t) + \sum_{\alpha=1}^A M^{(\alpha)}(t)\Delta t + \vartheta \left((\Delta t)^2 \right). \tag{B26} \\ \xi(t + \Delta t) = \xi(t) + \dot{\xi}(t)\Delta t + \vartheta \left((\Delta t)^2 \right) \\ = \xi(t) + \Xi(t)\Delta t + \vartheta \left((\Delta t)^2 \right) \end{aligned}$$

Iterating the procedure (B19)–(B26), one can calculate the whole process in the system, i.e., the sequence of states $Z(t), Z(t + \Delta t), Z(t + 2\Delta t), \dots$ etc. at given initial state $Z_0 = Z(t_0)$, free enthalpy function G and process functions $M^{(\alpha)} = 1 \dots A$, and Ξ . For numerical calculations, implicit methods of order $O(5)$ or higher are recommended.

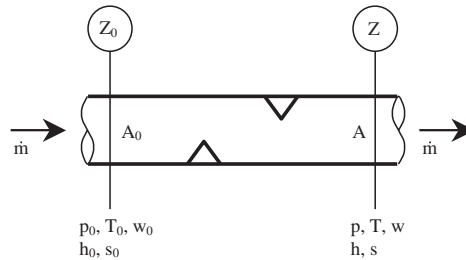


Figure 4 Stationary adiabatic flow of a fluid, i.e., liquid or gaseous medium in a tube. Initial state $Z_0(p_0, t_0, w_0, h_0, s_0)$, final state $Z(p, t, w, h, s)$. Mass flow $\dot{m} = \text{const}$.

The formalism presented above can also be used to analyze stationary states of the open system Σ . We will demonstrate this using a very simple example; namely, the adiabatic flow of a fluid, i.e., a liquid or gas, in a tube; Figure 4.

Let us consider a tube with varying cross section and some obstacles in it. A fluid of constant mass flow ($\dot{m} = \text{const}$) enters it somewhere at cross-section A_0 in an initial state (Z_0) characterized by its pressure, p_0 , temperature, T_0 , velocity, w_0 , specific enthalpy h_0 , and entropy, s_0 . A typical situation in engineering is that one has some information about the entrance state, Z_0 , for example (p_0, t_0, A_0) , and on the final state, Z , namely the pressure of the fluid, p , and cross section of the tube, A , at leaving, but neither the mass nor volume flow or the respective velocities are known. If the fluid's density and enthalpy are temperature and pressure dependent, according to the thermal equation of state (TEOS),

$$\rho_0 = \rho(p_0, T_0), \quad \rho = \rho(p, T), \quad (\text{B27})$$

and the caloric equation of state (CEOS),

$$h_0 = h(p_0, T_0), \quad h = h(p, T), \quad (\text{B28})$$

the balance equation of mass,

$$\dot{m} = A_0 \rho_0 w_0 = A \rho w, \quad (\text{B29})$$

and energy (we omit potential energy),

$$h_0 + \frac{1}{2} w_0^2 = h + \frac{1}{2} w^2, \quad (\text{B30})$$

do not allow us to calculate algebraically the temperature, T , and velocity, w , of the final state even if the mass flow is known; for example, by measuring the

entrance velocity w_0 and applying Eq. (B29). This is due to the fact that the energy balance (B30) includes both unknown quantities w and T . In order to resolve this situation let us consider the entropy balance of the tube considered to be an open thermodynamic system:

$$\dot{S}_T = \frac{\dot{Q}}{T^*} + (s_0 - s)\dot{m} + P_s. \tag{B31}$$

Here S_T is the (accompanying equilibrium) entropy of the fluid in the tube, \dot{Q} the heat exchanged between the tube and its surroundings, T^* is the temperature of the environment, and P_s is the entropy production of the fluid flow. As we assume adiabatic conditions $\dot{Q} = 0$. Also, due to the stationary condition, all extensive quantities of the fluid in the tube are constant, i.e., we have

$$\dot{m}_T = 0, \dot{U}_T = 0, \dot{S}_T = 0. \tag{B32}$$

Here m_T is the total mass of the fluid in the tube, U_T its internal energy, and S_T its entropy. Hence, Eq. (B31) can be rewritten as

$$P_s = (s - s_0) \dot{m} \geq 0, \tag{B33}$$

leading according to Eckart and Onsager to a process equation which in its most simple linear form is

$$\dot{m} = L(s - s_0), \quad L \geq 0. \tag{B34}$$

Here L is a phenomenological coefficient of the fluid flow depending on the geometry of the tube and the obstacles in it but also on the thermophysical properties of the fluid, i.e., its viscosity, etc. In practice, L has to be measured; but once it is known, it would allow us to calculate the mass flow in the tube in a certain range of pressure and temperature in the vicinity of the original data (p_0, T_0) .

The EOSs (B27), (B28), the balance equations (B29), (B30), and the process equation (B34) now can be combined to result in two equations:

$$h(p, T) - h(p_0, T_0) = \frac{1}{2} \left(\left(\frac{\dot{m}}{A_0 \rho_0} \right)^2 - \left(\frac{\dot{m}}{A \rho(p, T)} \right)^2 \right), \tag{B35}$$

$$\dot{m} = L (s(p, T) - s(p_0, T_0)), \tag{B36}$$

for the two unknown quantities (T, \dot{m}) if the equations of state (TEOS, CEOS), the related entropic equation of state (E EOS), $s = s(p, T)$, and the macroscopic transport coefficient (L) are known.

As an example, let us consider the adiabatic irreversible flow of an incompressible fluid with equations of state

$$T \text{ EOS} : \rho = \rho_0 = \frac{1}{v} = \text{const}, \quad (\text{B37a})$$

$$C \text{ EOS} : h = h_0 + c_p(T - T_0), c_p = \text{const}, \quad (\text{B37b})$$

$$E \text{ EOS} : s = s_0 + c_p \ln \left(\frac{T}{T_0} \right). \quad (\text{B37c})$$

These together with Eqs. (B35) and (B36) lead to a (transcendental) equation for the temperature, T , of the exit state:

$$T - T_0 = \frac{1}{2c_p} \left(\frac{1}{A_0^2} - \frac{1}{A^2} \right) \left(L \frac{c_p}{\rho} \ln \left(\frac{T}{T_0} \right) \right)^2 \quad (\text{B38})$$

This equation can be solved numerically by the iteration procedure:

$$T = \lim_{n \rightarrow \infty} T_n, \quad T_{n+1} = f(T_n), \quad T_0$$

with the symbol “f” indication the r.h.s. of Eq. (B38) for $T \rightarrow T_n$.

Finally we would like to mention that the process equation (B36) with Eq. (B37c) also can be rewritten as

$$T = T_0 \exp(\dot{m}/c_p L) \quad (\text{B39})$$

which for reversible flows, i.e., $L \rightarrow \infty$ results in the isothermal condition $T = T_0$. For more examples of thermodynamics of irreversible processes as applied to engineering systems, the reader is referred to [B3, B6, B9, B20, B25, B42, B47–B49, B54].

List of symbols

A	m^2 various	Cross-section area of a tube; also affinity-related to an internal variable
c_p	kJ/kgK	Specific isobaric heat capacity
G	kJ	Gibbs free enthalpy of a thermodynamic system
h	kJ/kg	Specific enthalpy
$h^{(\alpha)}$	kJ/kg	Specific enthalpy of mass flowing in or leaving an open system at position $\alpha = 1 \dots A$
L	$\text{kg}^2\text{K}/(\text{skJ})$	Phenomenological parameter of process equation of a fluid flow, cf. Eq. (B34)
\dot{m}	kg/s	Mass flow
n	mol	Mole number of material in a system
p	$\text{Pa} = \text{N/m}^2$	Pressure
p^*	$\text{Pa} = \text{N/m}^2$	Pressure in the environment of a system
P_s	kJ/Ks	Entropy production
\dot{Q}	$\text{kJ/s} = \text{kW}$	Heat flow
s	kJ/kgK	Specific entropy
S	kJ/K	Entropy of a system
T	K	Temperature
t	s	Time
T^*	K	Environmental temperature
U	kJ	Internal energy of a system
V	m^3	Volume of a system
w	m/s	Velocity
W	kJ	Volume-related work
Z, Z_0		Symbols for different thermodynamic states of a sys- tem or material
μ	kJ/kg	Chemical potential of material within a thermody- namic system
$\mu^{(\alpha)}$	kJ/kg	Chemical potential of mass flowing to or leaving an open thermodynamic system at position α ; cf. Figure 1
ρ	kg/m^3	Density of mass
Σ		Symbol for a thermodynamic system

Σ^*		Symbol for the surroundings of a thermodynamic system Σ
ξ	various	Internal variable

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C. Denaturation of proteins: Thermodynamic analysis

Proteins in their native or natural state are normally highly folded and densely packed. Also, they often exhibit remarkable structural stability against external disturbances and thus maintain their biological activities. However, when the environmental solution is subjected to substantial changes in temperature, pressure, or pH value, proteins may lose their native structure, i.e., transit from their natural state (N) to another denatured state (D), which often is characterized by partial or complete defolding of the protein’s amino-acid chains, thus inducing a quasi random structure of all side chains and atomic groups of the protein that is losing its bioactivity [C1, C11, C12].

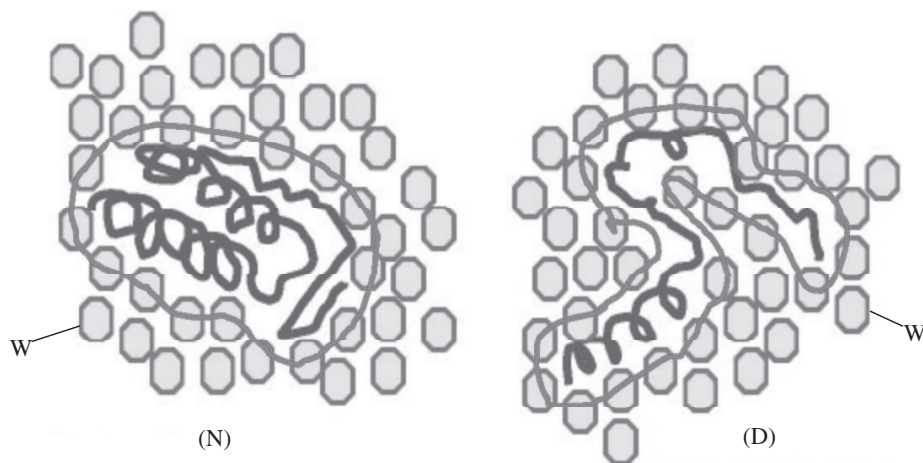


Figure 5 Protein in aqueous solution. N, native, compact state; D, denatured or unfolded state [C2]; W, water.

Though the molecular mechanism of this transition and also the molecular structure of the denatured state are often unknown, experience shows that the transition $N \rightarrow D$ sometimes is reversible. In these cases, the transition $N \rightarrow D$ may be considered as a quasi-chemical reaction leading to equilibrium between the proteins P_N in their native state (N) and those P_D in any kind of denatured state (D):



Hence, if we consider an aqueous solution of the protein at pressure, p , and temperature, T (cf. Figure 6), this equilibrium state can be characterized by a minimum of the Gibbs free enthalpy of the solution, i.e.,

$$G = G(n_N, n_D, n_W, n_H^+, p, T) \rightarrow \text{Min}, \quad (C2)$$

at constant values of $(n_p = n_D + n_N, n_W, n_H^+, p, T)$ [C3, C4].

Here, (n_N, n_D, n_W, n_H^+) indicate the mole numbers of protein in native state (N), in denatured state (D), the mole number of water and of free hydrogen ions or protons to take the pH value of the solution into account.

Introducing the reaction number or rate ξ of the chemical reaction ($P_N \rightarrow P_D$) by the relations

$$\begin{aligned} n_N &= n_{N0} - \xi \\ n_D &= n_{D0} + \xi, \end{aligned} \quad (C3)$$

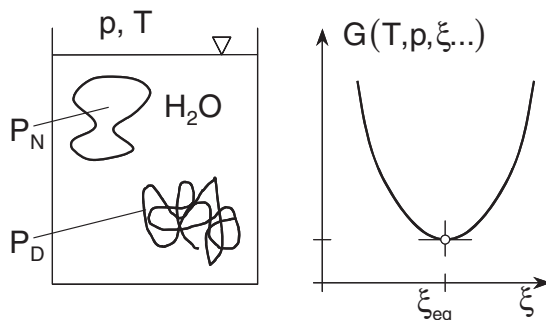


Figure 6 Aqueous solution of protein in a native (N) and a denatured (D) state. Gibbs free enthalpy G as a function of the rate number ξ of the quasi-chemical reaction $N \leftrightarrow D$ with equilibrium value ξ_{eq} .

i.e.,

$$dn_N = -dn_D = -d\xi, \quad (\text{C4})$$

we get from the Gibbs equation of the free enthalpy

$$dG = -SdT + Vdp + \mu_N dn_N + \mu_D dn_D + \mu_W dn_W + \mu_H^+ dn_H^+ \quad (\text{C5})$$

at

$$p = \text{const}, T = \text{const}, n_W = \text{const}, n_H^+ = \text{const},$$

the minimum conditions

$$\begin{aligned} dG &= (-\mu_N + \mu_D)d\xi = 0, \\ d^2G &= \left(\frac{\partial}{\partial \xi} (-\mu_N + \mu_D) \right) (d\xi)^2 > 0, \end{aligned} \quad (\text{C6})$$

i.e.,

$$\mu_N(\dots \xi_{\text{eq}} \dots) = \mu_D(\dots \xi_{\text{eq}} \dots), \quad (\text{C7})$$

$$\left(\frac{\partial \mu_D(\xi)}{\partial \xi} \right)_{p, T, \xi_{\text{eq}}} > \left(\frac{\partial \mu_N(\xi)}{\partial \xi} \right)_{p, T, \xi_{\text{eq}}}. \quad (\text{C8})$$

In Eq. (C3) the quantities (n_{N0}, n_{D0}) indicate the number of moles of protein in the solution at any initial state (Z_0). Also μ_x , $x = N, D, W, H^+$ in Eq. (C5) are the Gibbs chemical potentials of components ($x = N, D, W, H^+$).

From Eq. (C7) the equilibrium value of the reaction number

$$\xi_{eq} = \xi_{eq}(p, T, n_W, n_H^+, n_{D0}, n_{N0}), \tag{C9}$$

which is a function of (p, T, n_W, n_H^+) and of the initial values of the protein mole numbers (n_{D0}, n_{N0}) can be calculated in principle.

Inequality (C8) is necessary to hold for thermodynamic stability of the protein solution. Of course, it should be taken into account if analytical models of the chemical potentials (μ_N, μ_D) are considered.

Now, in pursuing the equilibrium condition (C7), we introduce the standard representation of the chemical potentials $\mu_i, i = N, D$ as

$$\mu_i = \mu_{i0}(p, T, n_W, n_H^+, n_i) + RT \ln(x_i \gamma_i), i = N, D. \tag{C10}$$

Here

$$\mu_{i0} = \mu_{i0}(p, T, n_W, n_H^+, n_i), i = N, D \tag{C11}$$

is the chemical potential of pure (P_N, P_D) in the solution (n_W, n_H^+) at (p, T) and

$$x_i = \frac{n_i}{n}, n = n_W + n_N + n_D, \tag{C12}$$

$$\gamma_i = \gamma_i(p, T, n_W, n_H^+, n_N, n_D,), i = N, D \tag{C13}$$

are the molar fraction and the “activity” of component $(i = D, N)$, respectively [C5]. For ideal solutions, we have $\gamma_i = 1$, but for real protein solutions $\gamma_i \neq 1$, $(i = D, N)$. Introducing Eq. (C10) into the equilibrium condition (C7), we get the law of mass action of the reaction (C1), i.e.,

$$K_{eq} = e^{-\Delta G_{DN}/RT}, \tag{C14}$$

with the definition of the equilibrium constant,

$$K_{eq} = \frac{\gamma_D^{x_D}}{\gamma_N^{x_N}}, \tag{C15}$$

and the difference of the chemical potentials of pure components (N, D) in the solution,

$$\Delta G_{DN} = \mu_{D0}(p, T, n_W, n_H^+, n_D) - \mu_{N0}(p, T, n_W, n_H^+, n_N) \quad (C16)$$

As this quantity in principle can be determined as a function of temperature (T) by microcalorimetric measurements [C6, C7], Eq. (C14) allows for ideal solutions, i.e., $\gamma_D = \gamma_N = 1$, to determine the equilibrium concentrations (x_N, x_D) of the protein in its native and denatured state, respectively, in the solution at a given temperature. An example for this will be given in the next paragraph. However, it should be emphasized that the assumption of ideality of a protein formulation ($\gamma_D = \gamma_N = 1$) is often only a rough approximation and may not hold in the actual system considered. In such a case, measurements of the concentrations of (P_D, P_N) should be carried out – for example, by applying appropriate spectroscopic methods [C8].

Also, denaturation of a protein may occur due to changes in pressure and the pH value of a formulation, i.e., possible instability of a protein against such changes also should be taken into account [C13].

The denaturation process itself can be described simply in thermodynamic terms by applying the method of thermodynamics of irreversible processes as outlined in Section B. Actually, we take advantage here of the isothermal and isobaric conditions at which the transition ($N \leftrightarrow D$) occurs, as in such processes the (negative) production term of the Gibbs free enthalpy and that of the entropy simply are related by Eq. (C5):

$$\dot{G} = -T\dot{P}_s \leq 0. \quad (C17)$$

Hence, in view of Eq. (C5) for the entropy production, we have

$$P_s = \frac{1}{T}(\mu_N - \mu_D)\dot{\xi} \geq 0. \quad (C18)$$

Following the principle of Eckard and Onsager, process equations for the “reaction” ($N \leftrightarrow D$) can be set up as linear relations between the thermodynamic flux ($\dot{\xi}$) and the related force $(\mu_N - \mu_D)/T$, i.e.,

$$\dot{\xi} = A_{ND}(\mu_N - \mu_D) + O(2). \quad (C19)$$

Here, the symbol “O(2)” indicates terms of higher order in powers of $(\mu_N - \mu_D)$.

For ideal protein components (N, D) of the broth, this equation can also be rewritten as

$$\dot{\xi}(t) = A_{ND}RT \ln \left(\frac{x_N x_{DE}}{x_{NE} x_D} \right), \quad (C20)$$

with x_N , x_D being the actual molar concentrations of the native and the defolded protein realized in the fluid, and x_{NE} , x_{DE} the respective concentrations of the proteins at given total amount ($n_N + n_D = \text{const}$) which are determined by the law of mass action (C14), (C16). The argument in the logarithm obviously deviates from one as long as $x_N \neq x_{NE}$ or $x_D \neq x_{DE}$. Hence, it may be considered as a measure for the deviation of the protein solution from equilibrium, actually determining how fast – if at all – equilibrium is reached. The phenomenological coefficient ($A_{ND} \geq 0$), cf. Eq. (C18), in practice only can be determined from time series measurements of the concentrations $x_N(t)$, $x_D(t)$. It should be noted that A_{ND} basically depends on the temperature (T) and on the pressure (p) in the solution and possibly also on additional parameters such as the pH value or other chemical components in the solution. For more details, see the (ever growing) literature [C9, C10].

Thermal stability of myoglobin

Myoglobin is a medium-sized protein consisting of a chain of 153 amino acids including a protoporphyrin IX ring with an iron atom as its active center. The amino acids form 8 α -helices surrounding the center, leading in native state to a nearly ellipsoidal form of the protein with diameters $44 \times 44 \times 25$ (Å)³. The summation formula of myoglobin is



The molar mass of myoglobin is $M = 17.053$ g/mol. The sequence of amino acids and stereo diagrams thereof can be found in [C1, p. 167]. Myoglobin can be found in all oxygen-consuming living systems. It is a part of the oxygen supply system for the heart and other muscles of mammals and it serves as a carrier and storage system for oxygen in biological tissues and cells. There its normal concentration is $c < 100$ $\mu\text{mol/l}$.

Myoglobin is very sensitive to changes in temperature, i.e., it easily can be defolded or denatured at both high and low temperatures. This can severely change its biological activity. To be more specific, we present experimental data of the change in the free enthalpy; cf. Eq. (C16),

$$\Delta G_{DN} = \mu_{D0} - \mu_{N0},$$

of myoglobin formulations including only denatured (μ_{D0}) or native (μ_{N0}) protein at same pressure ($p = 1$ bar), temperature ($270 \text{ K} < T < 340 \text{ K}$), and pH value; see Table 1, [C14]. These data have been determined by microcalorimetric and spectroscopic measurements [C6, C9]. They are complemented by the respective data for the enthalpy and entropy differences of these states to be calculated from ΔG_{DN} by the standard relations

$$\Delta H_{DN} = H_{D0} - H_{N0} = \Delta G_{DN} - T \left(\frac{\partial \Delta G_{DN}}{\partial T} \right)_p, \quad (\text{C21})$$

$$\Delta S_{DN} = S_{D0} - S_{N0} = - \left(\frac{\partial \Delta G_{DN}}{\partial T} \right)_p. \quad (\text{C22})$$

Here, (H_{i0} , S_{i0} , $i = D, N$) indicate the partial molar enthalpies and entropies of the myoglobin formulations, including either denatured protein ($i = D$) or native protein ($i = N$) only.

Table 1 Thermodynamic data of the differences in the free enthalpy ΔG_{DN} , enthalpy ΔH_{DN} , and entropy ΔS_{DN} as functions of temperature, T , of formulations including the protein only in either a denatured (D) or native state (N) at constant pressure, $p = 1$ atm. For definitions of (ΔG_{DN} , ΔH_{DN} , ΔS_{DN}), cf. Eqs. (C16), (C21), (C22) and [C14].

T/K	270	280	290	300	310	320	330	340
ΔG_{DN} (kJ/mol)	-3.16	5.13	11.8	15	15.8	11.8	5.13	-3.53
ΔH_{DN} (kJ/mol)	-289.8	-204	-115	-23	72.2	170.4	271.8	376.4
ΔS_{DN} (kJ/mol K)	-1.066	-0.754	-0.442	-0.13	0.182	0.494	0.806	1.118

The ΔG_{DN} data of Table 1 can be simply correlated by a polynom of second order as

$$\Delta G_{DN}(T) = A_2 T^2 + A_1 T + A_0, \quad (\text{C23})$$

with

$$\begin{aligned} A_2 &= 1.56 \cdot 10^{-2} \text{kJ/molK}^2, \\ A_1 &= 9.49 \text{kJ/molK}, \\ A_0 &= -1427 \text{kJ/mol}. \end{aligned} \quad (\text{C24})$$

This function and data of Table 1 are sketched in Figure 7a. Also, numerical data of ΔH_{DN} and $T \cdot \Delta S_{DN}$ are shown in Figure 7b as a function of the

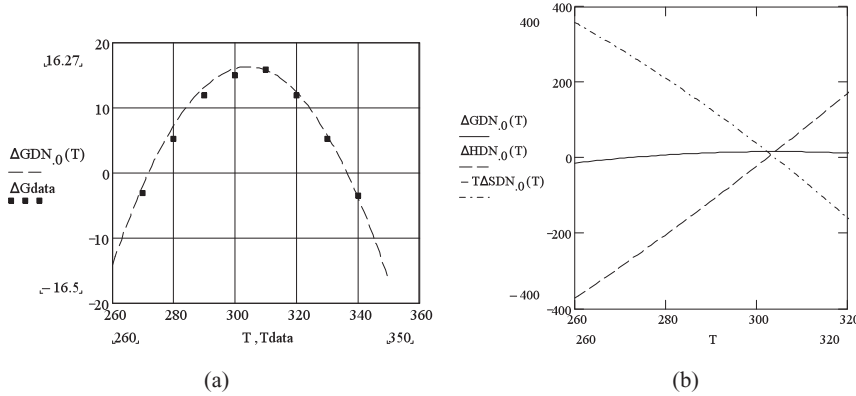


Figure 7 (a) Free enthalpy differences ($\Delta G_{DN}/(\text{kJ/mol})$) versus temperature (T/K) data of myoglobin formulations presented in Table 1 and correlation function (C23), (C24). This figure allows the determination of the ranges of temperature in which the myoglobin is thermally stable ($\Delta G_{DN} > 0$) or will be unstable ($\Delta G_{DN} < 0$). (b) Enthalpy differences ($\Delta H_{DN}/(\text{kJ/mol})$) and entropy ($-T \cdot \Delta S_{DN}/(\text{kJ/mol})$) difference data calculated from ΔG_{DN} data via the correlation function (C23), (C24), i.e., by Eqs. (C21a) and (C22a). Enthalpic stability of the formulation is given for temperature ranges with $\Delta H_{DN}(T) > 0$, i.e., for $T > 302.4 \text{ K}$. Entropic stability of the formulation is given if $\Delta S_{DN} > 0$, i.e., for temperatures $T < 304.2 \text{ K}$. Therefore, enthalpic as well as entropic stability is only possible in the small temperature range ($302.4 \text{ K} < T < 304.2 \text{ K}$) or ($29.3 \text{ }^\circ\text{C} < T < 31.0 \text{ }^\circ\text{C}$).

system's temperature (T). These data simply can be calculated by using the correlation (C23) and the relations (C21) and (C22), leading to

$$\Delta H_{DN} = -A_2 T^2 + A_0, \tag{C21a}$$

$$\Delta S_{DN} = -A_2 T - A, \tag{C22a}$$

In a thermodynamic system at constant pressure, p , and temperature, T , changes can only occur if the free enthalpy of the final state is smaller than that of the initial state, so one can recognize that the myoglobin formulation will be thermally stable for

$$\Delta G_{DN} > 0 \quad \text{or} \quad \mu_{D0} > \mu_{N0}, \tag{C25}$$

but unstable for

$$\Delta G_{DN} < 0 \quad \text{or} \quad \mu_{D0} < \mu_{N0}. \tag{C26}$$

Accordingly, the temperatures for which

$$\Delta G_{DN}(T) = 0 \tag{C27}$$

will be transition points separating temperature regions of thermal stability and instability of the myoglobin formulation from each other. Using the correlation function (C23) and data (C24), condition (C27) leads to the “transition temperatures,” cf. Figure 7a,

$$\begin{aligned} T_{10} &= 336.5\text{K} = 63.3\text{ }^\circ\text{C}, \\ T_{20} &= 271.86\text{K} = -1.3\text{ }^\circ\text{C}; \end{aligned} \quad (\text{C27a})$$

that is, the formulation is stable for $T_{20} < T < T_{10}$, otherwise unstable.

Assuming the myoglobin formulation to be an ideal solution, i.e., $\gamma_D = \gamma_N = 1$, we can infer from the law of mass action (C14), (C15) that at T_{10} , T_{20}

$$x_D(T_{i0}) = x_N(T_{i0}) = \frac{1}{2}, \quad i = 1, 2; \quad (\text{C28})$$

that is, at these temperatures the formulation includes equal mole numbers of myoglobin in its native and (any kind of) denatured state.

However, the ideality assumption introduced above is very dissatisfying. Indeed, one can avoid it and use a much weaker assumption. For this, let us consider the temperature at which ΔG_{DN} has its maximum value:

$$\left(\frac{\partial \Delta G_{DN}}{\partial T} \right)_{p, T_{\max}} = -\Delta S_{DN}(T_{\max}) = 0. \quad (\text{C29})$$

Numerically, we get from (C19), (C20): $T_{\max} = 304.17\text{ K} = 31.1\text{ }^\circ\text{C}$. Now, as the entropy of the myoglobin in the formulation according to Eq. (C22) at T_{\max} is equal in both the native (N) and the denatured (D) state, these can only differ in their enthalpies. Indeed, we have from Eqs. (C21) and (C21a):

$$\Delta H_{DN}(T_{\max}) = H_{D0}(T_{\max}) - H_{N0}(T_{\max}) = 16.27\text{ kJ/mol}. \quad (\text{C30})$$

Considering a mixture of D and N myoglobin at T_{\max} , the occupation numbers – or molar fractions (x_D , x_N) – in both states should be determined by a simple Boltzmann distribution; i.e., we have in this state

$$\left(\frac{x_D}{x_N} \right)_{T_{\max}} = \exp \left(-\frac{\Delta H_{DN}(T_{\max})}{RT_{\max}} \right) \quad (\text{C31})$$

or, in view of Eq. (C16),

$$\left(\frac{x_D}{x_N}\right)_{T_{\max}} = 1.606 \cdot 10^{-3}. \quad (\text{C31a})$$

Hence, we can determine the ratio of the activity coefficients (γ_D/γ_N) in this state from the law of mass action (C14), (C15) as the difference in the free enthalpies of the D and the N state at (T_{\max}), as $\Delta G_{DN}(T_{\max})$ is known from Eqs. (C23) and (C24):

$$\ln\left(\frac{\gamma_D}{\gamma_N}\right)_{T_{\max}} + \ln\left(\frac{x_D}{x_N}\right)_{T_{\max}} = -\Delta G_{DN}T_{\max}/(RT_{\max}). \quad (\text{C32})$$

Actually, with $\Delta G_{DN}(T_{\max}) = 17.06$ kJ/mol and in view of Eq. (C31a), we obtain

$$\left(\frac{\gamma_D}{\gamma_N}\right)_{T_{\max}} = 0.731. \quad (\text{C33})$$

We now assume this ratio to be constant in the temperature range considered in Figure 7a, i.e., for ($260 \text{ K} < T < 350 \text{ K}$). Then we can determine the molar fractions of the D and N form of myoglobin at the transition temperatures ($T_{10} = 336.5 \text{ K}$, $T_{20} = 271.8 \text{ K}$) for thermal stability, i.e., when $\Delta G_{DN} = 0$. As in this case – cf. Eq. (C14) –

$$\left(\frac{\gamma_D x_D}{\gamma_N x_N}\right)_{T_{i0}} = 1, \quad i = 1, 2 \quad (\text{C34})$$

we obtain with (C33), $x_D(T_{i0}) = 0.58$, $x_N(T_{i0}) = 0.42$, $i = 1, 2$.

This result demonstrates that the ideality assumption ($\gamma_D = \gamma_N = 1$) is not too bad after all, as it would lead to $x_{D\text{id}}(T_{i0}) = x_{N\text{id}}(T_{i0}) = 0.5$.

Finally, we want to determine the temperature range in which the myoglobin formulation should be stored if a concentration of native (N) and denatured (D) protein of $x_N \geq 0.99$, $x_D \leq 0.01$ should be maintained. Again, the law of mass action (C14) with Eqs. (C15), (C23), and (C24) and the ratio of the activity coefficients (C33) lead to an algebraic equation for the limiting temperatures:

$$A_2 T^2 + A_1 T + A_0 = -RT \ln\left(\frac{\gamma_D x_D}{\gamma_N x_N}\right), \quad (\text{C35})$$

leading to the temperature interval

$$287.1\text{K} < T < 318.7\text{K}$$

or ($14^\circ\text{C} < T < 45.5^\circ\text{C}$). This information certainly would support pharmacists in their efforts to store myoglobin formulations at the appropriate temperatures during their “shelf lives” in order to avoid heat- or cold-induced denaturation and the concomitant loss of biological or pharmaceutical activity.

List of symbols

A_{ND}	$\frac{\text{mol}^2}{\text{J}_s}$	Phenomenological coefficient of the (N \leftrightarrow D) transition process
D	–	Symbol for the denatured or (often) defolded state of a protein
G	J	Gibbs free enthalpy of an aqueous solution of proteins
N	–	Symbol for the native or natural state of a protein
$n = n_{\text{w}} + n_{\text{D}} + n_{\text{N}}$	mol	Total number of moles of protein in (N, D) states, respectively, and of water in an aqueous protein solution
n_{D}	mol	Number of protein in a denatured state in an aqueous solution
$n_{\text{D}0}$	mol	Mole number of denatured protein in an initial state (Z_0) of a protein–water solution
n_{H}^+	mol	Mole number of hydrogen ions (protons) in an aqueous protein solution (pH value)
n_{N}	mol	Mole number of native protein in an aqueous solution
$n_{\text{N}0}$	mol	Mole number of native protein in an initial state (Z_0) of a protein–water solution
$n_{\text{p}} = n_{\text{D}} + n_{\text{N}}$	mol	Total mole number of protein in an aqueous solution
n_{w}	mol	Number of moles of water in an aqueous protein solution
P	Pa	Pressure

P_s	J/Ks	Entropy production of the denaturation process of a protein in aqueous solution
S	J/K	Entropy of an aqueous solution of proteins
T	K	Temperature
V	m^3	Volume of an aqueous solution of proteins
$x_i = n_i/n$	1	Molar fraction of component ($i = D, N, W$) of a protein–water solution
Z_0	–	Symbol for an initial state of a protein–water solution
γ_i	1	Activity coefficient of the chemical potential of component ($i = D, N, W$) of a protein–water solution
$\Delta G_{DN} = \mu_{D0} - \mu_{N0}$	J/mol	Change of the molar free enthalpy of the denatured proteins in solution upon complete renaturation in the solution at (p, T)
$\Delta H_{DN} = H_{D0} - H_{N0}$	J/mol	Change of the molar enthalpy of the denatured proteins in solution upon complete renaturation in the solution at (p, T)
$\Delta S_{DN} = S_{D0} - S_{N0}$	J/mol	Change of the molar entropy of the denatured proteins in solution upon complete renaturation in the solution at (p, T)
μ_D	J/mol	Chemical potential of denatured protein in a solution
μ_N	J/mol	Chemical potential of native protein in a solution
μ_W	J/mol	Chemical potential of water in a protein–water solution
ξ	mol	Reaction number of the quasi-chemical transition of a protein from a native state (N) to a denatured state (D)
ξ_{eq}	mol	Value of the reaction number (ξ) in an equilibrium state of the protein–water solution

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D. Hydration of proteins

Complex biochemical molecules like proteins, enzymes, polyelectrolytes, biopolymers, etc., in aqueous solution exhibit quite complex interaction phenomena with the surrounding water molecules [D1–D3]. The basic reason for this is that the surface of most biomolecules includes polar atomic groups or even local electrical charges, providing sites where other polar molecules like water, alcohols, etc., may be adsorbed. The number of water molecules included in the first layer coating a biomolecule is about 10^2 – 10^5 . For example, to cover the ellipsoidal surface of the protein myoglobin at normal conditions with a mono-layer of water, 800–900 water molecules are needed; cf. Section C (“Denaturation of proteins”) and [D17]. However, it should be emphasized that for a protein to exhibit its full biological activities or enzymatic properties, a mono-layer of water is not needed at all but rather a percolation-like network of water molecules allowing transport by interactions between

themselves and with the surface atoms of the protein energy and information between different parts of the surface of the protein [D14].

The water adsorbed may penetrate or intrude into the biomolecule and thus eventually change its (higher order) structure and also change the number of adsorption sites for other water molecules. Indeed, it is sometimes appropriate to distinguish water molecules within caverns or shells formed within the tertiary structure of the protein from those being adsorbed on the outer or inner “surface” of the protein, i.e., basically being adsorbed on the “helices” and “sheets” of the protein [D16].

Also it should always be taken into account that the structure and properties of a protein–water system always depend on temperature, T , and pressure, p , of its (aqueous) environment. Roughly speaking, it can be said that proteins at high temperatures unfold completely and thus lose their “cavern-water;” whereas at high pressures the water intrudes more and more into the protein, which swells and finally transfers to a highly hydrated and swollen state, thus losing most of its tertiary or even secondary structure. Knowledge about this can be gained by either experimental methods like NMR or microwave spectroscopy and also – to a certain extent – by numerical simulation or “in silico” experiments [D15, D16].

The adsorbed water molecules also may form a network or a shell that may compress or deform the protein (adsorption compression [D4]). Both effects may change the bioactivity of the biomolecule considered.

Conversely, proteins and other much smaller biomolecules change the structure of the network and also the states of single water molecules in their surroundings compared to the corresponding properties of water molecules in the bulk phase. An interesting example for this was given recently by M. Havenithor in studies of lactose–water solutions using Terahertz spectroscopy. Havenithor demonstrated that the mobility of water molecules in the vicinity of a lactose molecule was reduced, i.e., the water molecules moved much slower than in the bulk phase. Also, the rotational frequencies of these quasi-adsorbed water molecules were reduced by approximately 30% [D5], the energy thereby lost seemingly being stored in the many internal degrees of freedom of the lactose molecule.

Though the molecular mechanism of the water–biomolecule interactions often is not known in detail, this phenomenon can be described by a thermodynamic formalism, at least as long as it can be considered as a collective phenomenon occurring in aqueous solutions where the concentration of the biomolecule considered is, say, at least 1 n mol/liter.

The key concept to do this is that of an “internal variable” (ξ), which is assigned to the biomolecule and which characterizes its actual state in an aqueous solution. To be specific, we will understand (ξ) to be the number of sites on the external or internal surface of the biomolecule where water molecules can be adsorbed (in one or more layers). Also, we assume henceforth that the biomolecule considered is a compact protein with a certain number (ξ_0) of adsorption sites available for water molecules in a well-known reference state. Of course, the concept of “adsorbed water molecules” should be specified or elucidated from a molecular thermodynamic point of view. However, for the sake of generality, we refrain here from this and only assume that a water molecule is considered to be in an “adsorbed state” if it is in the vicinity of the atoms of the biomolecule such that its physical state is changed considerably compared to that in the surrounding bulk liquid phase.

Naturally, in order to get more detailed and, hopefully, more accurate phenomenological descriptions of protein–water systems, not one but several internal variables (ξ_1, ξ_2, \dots) of this system can be introduced. For example, one can distinguish the external “surface” of the protein from its internal cavities or pores, which may also include water molecules in states stimulated or changed by interactions with the surrounding atoms of the biomolecule. Actually, these “internal” water molecules can contribute to the so-called proton conductivity of the biomolecule and hence are highly important for transmitting electrical signals in the aqueous solution considered [D1].

Let us now consider a protein, P, the surface of which bears polar groups and electrical charges on which water molecules (w^a) with mole number n^a per mole of protein and chemical potential, μ^a , are adsorbed. We assume thermodynamic equilibrium to be established between the adsorbed water and the water (w^f) in the surrounding liquid phase with mole number n^f per mole of protein, and chemical potential, μ^f , at constant temperature, T, and external pressure, p, of the system.

We consider the protein molecule coated by water as a thermodynamic system and introduce the Gibbs free enthalpy of 1 mol of proteins as

$$G_m = G_m(T, p, n^a, \xi), \quad (\text{D1})$$

with the Gibbs equation [D6–D8]

$$dG_m = -S_m dT + V_m dp + \mu^a dn^a - A d\xi. \quad (\text{D2})$$

Here (S_m) is the molar entropy, (V_m) the molar volume of the protein–water adsorbate system. The parameter (A) indicates the so-called activity of the

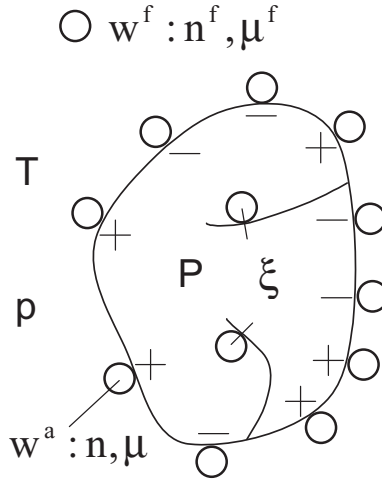


Figure 8 Scheme of a protein molecule with polar groups and electrical charges on its surface where water molecules with mole number n^a may adsorb from a bulk water phase in liquid state of mole number n^f .

internal variable (ξ). It can be considered as a kind of internal pressure of the protein, which is high in a compact state, but low in an unfolded, open configuration of the protein.

If the potential function (D1) is known explicitly, the various equations of state (EOS) of the protein–water system can be derived from it by simple differentiations.

The entropic-caloric equation of state (EEOS) is

$$-S_m = -S_m(T, p, n^a, \xi) = \left(\frac{\partial G}{\partial p} \right)_{pn\xi} . \tag{D3}$$

The thermal or volumetric equation of state (TEOS) is

$$V_m = V_m(T, p, n^a, \xi) = \left(\frac{\partial G}{\partial p} \right)_{Tn\xi} . \tag{D4}$$

The chemical or adsorption equation of state (EEOS) is

$$\mu^a = \mu^a(T, p, n^a, \xi) = \left(\frac{\partial G_m}{\partial \xi} \right)_{Tp\xi} . \tag{D5}$$

The internal equation of state (IEOS) is

$$-A = -A(T, p, n^a, \xi) = \left(\frac{\partial G_m}{\partial \xi} \right)_{T, p, n^a} \quad (D6)$$

A simple example for these equations of state (EOSs) will be given below.

Let us now consider two different types of equilibria that can be realized in the protein–water system characterized only by a simple potential function (D1) and the free enthalpy function of the bulk water phase:

$$G^f = G^f(P, T, n^f). \quad (D7)$$

Here n^f is the mole number of water in the liquid bulk phase per mole of protein. The total amount of water in the system per mole of protein is

$$n_w = n^f + n^a = \text{const.} \quad (D8)$$

It is considered to be a constant.

Assuming the exchange process of water between the bulk phase and the adsorbed phase ($n^f \leftrightarrow n^a$), especially its approach to “external” equilibrium to be a rapid process compared to the “conformational process” of the protein, i.e., its adaptation to a new “internal” equilibrium configuration caused by the water shells on its surface, we can distinguish equilibria states as follows:

a) Restricted or external-only equilibrium states

These are defined as external adsorption equilibria between the bulk phase and the adsorbed phase of water ($n^f \leftrightarrow n^a$) at frozen conformation of the protein, i.e., at constant value of the internal variable ξ or – normally – internal non-equilibrium characterized by non-vanishing of the affinity ($A \neq 0$), (D6). These states are according to the Second Law characterized by a minimum of the total free enthalpy of the system at constant values of pressure, p , temperature, T , and number of adsorption sites or protein conformation, ξ :

$$\begin{aligned} G &= G^f(p, T, n^f = n_w - n^a) + G_m(p, T, n^a, \xi) \rightarrow \text{Min} \\ T &= \text{const}, \quad p = \text{const}, \quad \xi = \text{const}, \quad n_w = \text{const} \end{aligned} \quad (D9)$$

This implies due to Eq. (D8), cf. [D7, D13],

$$\left(\frac{\partial G}{\partial n^a}\right)_{p,T,\xi} = 0, \left(\frac{\partial^2 G}{\partial n^{a2}}\right)_{p,T,\xi} > 0. \tag{D10}$$

Using the Gibbs equation for the bulk water phase (G^f), we have

$$dG^f = -S^f dT + V^f dp + \mu^f dn^f. \tag{D11}$$

Hence, from Eqs. (D2), (D10), and (D11) in view of Eq. (D9), we get the conditions of stable adsorption equilibria:

$$\mu^f(T, p) = \mu^a(T, p, n^a, \xi), \tag{D12}$$

$$\left(\frac{\partial \mu^a}{\partial n^a}\right)_{T,p,\xi} > 0. \tag{D13}$$

Condition (D12) is an algebraic equation that allows us to calculate the amount of water adsorbed on the protein at given parameters (p, T) and constant conformation (ξ):

$$n^a = n^a(p, T, \xi) \tag{D14}$$

This relation is known as the ‘‘adsorption isotherm’’ of water on the protein’s surface at frozen conformation, several algebraic expressions being explicitly known for liquid adsorption processes in the literature [D9, D10], cf. also Eq. (D38).

b) Full equilibrium states

These are defined as states realized at external adsorption equilibrium ($n^f \leftrightarrow n^a$) and conformational or internal equilibrium: $\xi = \xi_E$ of the structure of the protein and the surrounding water. These states are characterized by a minimum of the total free enthalpy of the system at constant pressure, p , and temperature, T :

$$\begin{aligned} G &= G^f(p, T, n^f = n_w - n^a) + G_m(p, T, n^a, \xi) \rightarrow \text{Min} \\ T &= \text{const}, \quad p = \text{const}, \quad n_w = \text{const} \end{aligned} \tag{D15}$$

Hence the equilibrium conditions are

$$\left(\frac{\partial G}{\partial n^a}\right)_{T,p,\xi} = 0, \left(\frac{\partial G}{\partial \xi}\right)_{T,p,n} = 0, \tag{D16}$$

$$\left\| \frac{\partial^2 G}{\partial n^a \partial \xi} \right\| > 0. \quad (\text{D17})$$

In view of (D2) and (D11) conditions given by Eq. (D16) can be rewritten as

$$\mu^a(p, T, n^a, \xi) - \mu^f(p, T) = 0, \quad (\text{D18})$$

$$A(p, T, n^a, \xi) = 0. \quad (\text{D19})$$

These are two algebraic equations from which the equilibrium values of the amount of water adsorbed (n_E^a) and the equilibrium conformation (ξ_E) in principle can be determined as functions of pressure, p , and temperature, T , i.e.,

$$n_E^a = n_E^a(p, T, n_w), \quad (\text{D20})$$

$$\xi_E = \xi_E(p, T, n_w). \quad (\text{D21})$$

Inequality (D17) is the stability condition for the equilibrium state $Z_E(p, T, n_E^a, \xi_E)$. If it does not hold, the state of the protein is thermodynamically unstable and spontaneous changes of both the amount of water adsorbed and the structure or conformation of the protein may occur at certain limiting values of pressure and temperature (p, T), which can be calculated from the condition, cf. [D7] and [D13],

$$\left\| \frac{\partial^2 G}{\partial n^a \partial \xi} \right\|_{pT} = 0. \quad (\text{D17a})$$

We now consider the behavior of protein–water systems in the vicinity of their total equilibrium state at given pressure, p , and temperature, T , of the system; i.e., configuration $\xi_E(p, T)$ and amount of water adsorbed $n_E^a(p, T)$. Then the total free enthalpy of the system

$$G = G^f + G_m \quad (\text{D22})$$

can be approximated by a Taylor series expansion of the second order, cf. Fig. 9, i.e.,

$$\begin{aligned} G(p, T, n^a, \xi) &= G_{00} + G_{10}(n^a - n_E^a) + G_{01}(\xi - \xi_E) \\ &+ \frac{1}{2} [G_{20}(n^a - n_E^a)^2 + 2G_{11}(n^a - n_E^a)(\xi - \xi_E) \\ &+ G_{02}(\xi - \xi_E)^2] + O(3) \end{aligned} \quad (\text{D23})$$

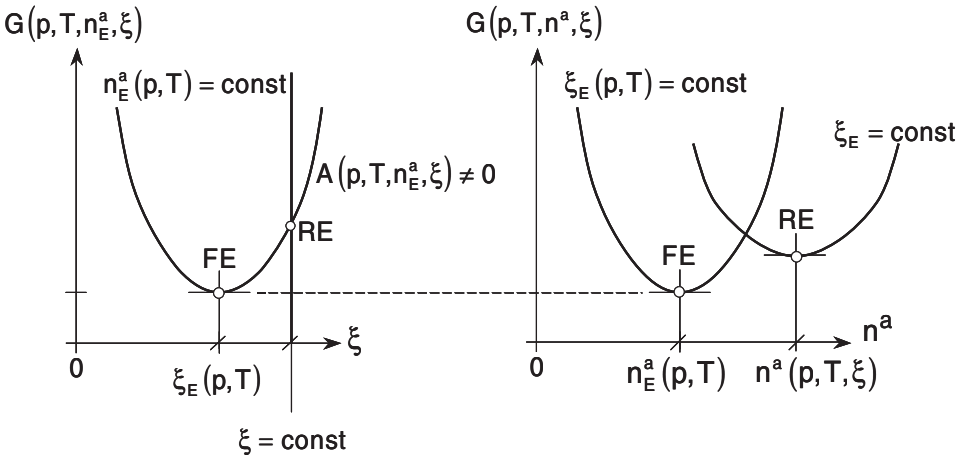


Figure 9 Gibbs free enthalpy of the protein–water system, cf. Figure 8 and Eqs. (D9), (D15). FE: $n_E^a = n_E^a(p, T, n_w), \xi_E = \xi_E(p, T, n_w)$ stands for full or unrestricted equilibrium. RE: $n^a = n^a(p, T, n_w, \xi), \xi = \text{const}$ stands for restricted adsorption equilibrium at frozen protein conformation ($\xi = \text{const}$).

Here, an arbitrary set of data (n^a, ξ) will refer to a non-equilibrium state in the vicinity of the reference equilibrium state (n_E^a, ξ_E) . The symbols $G_{ik}, i, k, = 0, 1, 2$, indicate the respective derivatives of the function $G(p, T, n^a, \xi)$ taken at the equilibrium state $Z_E(n_E^a(p, T), \xi_E(p, T))$.

In view of Eq. (D16), we have

$$G_{10} = \left(\frac{\partial G}{\partial n^a} \right)_{T, p, \xi_E} = 0, \quad G_{01} = \left(\frac{\partial G}{\partial \xi} \right)_{T, p, n_E^a} = 0. \tag{D24}$$

The stability condition (D17) leads to the inequalities

$$G_{20} > 0, G_{20}G_{02} - G_{11}^2 > 0, G_{02} > 0. \tag{D25}$$

The chemical equation of state is, according to Eqs. (D5), (D11), (D23):

$$-\mu^f + \mu^a = G_{20}(n_a - n_E^a) + G_{11}(\xi - \xi_E) + O(2). \tag{D26}$$

Likewise, from Eqs. (D6), (D23) for the internal equation of state, we obtain

$$-A(p, T, n^a, \xi) = G_{11}(n^a - n_E^a) + G_{02}(\xi - \xi_E) + O(2), \tag{D27}$$

which, of course, is only the Taylor series expansion of the affinity (A) in its variables (n^a, ξ) at constant (p, T).

We now restrict ourselves to consider states with internal equilibrium only. These may be externally, i.e., as far as adsorption of water on the surface of the protein is concerned, non-equilibrium or – trivially – equilibrium states. The affinity (A) of the system must vanish in these states. Hence from Eq. (D27) we have

$$0 = G_{11}(n_a - n_E^a) + G_{02}(\xi - \xi_E), \quad (\text{D28})$$

i.e., the amount of water adsorbed, n^a , and the structure of the protein, ξ , represented for example by the number of adsorption sites, ($n^s \simeq \xi$), are linearly related for all non-equilibrium states of this type. Inserting Eq. (D28) into Eq. (D26), we get the non-equilibrium isotherm of the water adsorption process

$$n^a - n_E^a = H(\mu^a - \mu^f), \quad (\text{D29})$$

with the Henry constant

$$H = \frac{G_{02}}{G_{20}G_{02} - G_{11}^2} > \frac{1}{G_{20}} > 0. \quad (\text{D30})$$

Considering now another isothermal reference equilibrium state, $Z_E^+(n_E^{a+} = n_E^a(p^+, T), \xi_E^+ = \xi_E(p^+, T))$, Eq. (D29) can be rewritten as

$$n^a - n_E^{a+} = H^+(\mu^a - \mu^{f+}), \quad (\text{D31})$$

with (n_E^{a+}) being the equilibrium load of water on the protein's surface at the reference state Z_E^+ and H^+ the Henry's constant in this state.

Assuming the Henry's constants for both reference states (Z_E, Z_E^+) to be the same, i.e., $H = H^+$, we obtain, by substituting Eq. (D29) from Eq. (D31),

$$n_E^a - n_E^{a+} = H(\mu^f - \mu^{f+}), \quad (\text{D32})$$

or, in view of the standard representation of chemical potentials by fugacities (f),

$$\mu^f(p, T) = \mu^f(p^+, T) + RT \ln \left(\frac{f(p, p^+, T)}{p^+} \right), \quad (\text{D33})$$

$$n_E^a - n_E^{a+} = \text{HRT} \ln \left(\frac{f(p, p^+, T)}{p^+} \right). \quad (\text{D34})$$

This is the most simple form of the adsorption isotherm of water on proteins for full or external and internal equilibrium states. Note that it only includes the fugacity of the bulk water at system's pressure and temperature, p , T , referred to as the reference pressure (p^+). The protein is only presented by its Henry constant H (p^+ , T), which however may depend on the system's temperature, T .

Note that the ratio of the Henry's constants (H_1/H_2) of two different proteins (1, 2) can be calculated from $2 \times 2 = 4$ water adsorption experiments. Indeed, we obtain from Eq. (D34) written for both proteins (1, 2), by dividing the resulting equations by each other, the relation

$$\frac{(n_E^a - n_E^{a+})_1}{(n_E^a - n_E^{a+})_2} = \frac{H_1}{H_2}. \quad (\text{D35})$$

A numerical example for this relation referring to myoglobin and other proteins is in preparation and will be reported later on.

The chemical and the internal equation of state (D26) and (D27) include three constants (G_{20} , G_{11} , G_{02}) which normally are unknown and hence have to be determined for practical use. To achieve this, one has to introduce a model for the chemical potential of the adsorbed phase (μ^a), which, as already mentioned above, is not in its equilibrium state at given p , T . A simple class of such models can be described by the relation

$$\mu^a(p, T, n^a) = \mu^f(p, T) + RT \ln \left(\frac{p^a(n^a, \xi, T)}{p} \right). \quad (\text{D36})$$

Here, the function $p^a = p^a(n^a, \xi, T)$ indicates the isobar related to the equilibrium adsorption isotherm (AI) of the system,

$$n_E^a = n_E^a(p, T, \xi), \quad (\text{D37})$$

but taken for the non-equilibrium value n^a of the amount of water adsorbed and the actual conformation parameter, ξ , and pressure, p , in the system.

For example, if we choose for the adsorption isotherm (D37), the so-called Freundlich isotherm [D11],

$$n_E^a(p, T, \xi) = H_E(T) \cdot \xi_E \cdot p, \quad (\text{D38})$$

with a temperature-dependent Henry's constant (H_E) and a conformation parameter (ξ_E) that is assumed to be proportional to the number of adsorption sites for water on the protein, we get by inverting this equation

$$p(n_E^a, T, \xi) = \frac{n_E^a}{H_E(T) \cdot \xi_E}. \quad (\text{D39})$$

This is the pressure that is needed for a Freundlich adsorbate to have a number n_E^a of water molecules in full equilibrium adsorbed on the protein, which also is in a stable or equilibrium conformation ξ_E .

Considering now an arbitrary equilibrium number of adsorbed water molecules, n^a , and conformation, ξ , we can assign it a virtual pressure by the similar relation

$$p^a(n^a, T, \xi) = \frac{n^a}{H_E(T) \cdot \xi}, \quad (\text{D40})$$

which inserted into Eq. (D36) gives an explicit expression for the chemical potential of the adsorbed phase in the non-equilibrium state considered:

$$\mu^a(p, T, n^a) = \mu^f(p, T) + RT \ln \left(\frac{n^a}{H_E(T) \xi p} \right). \quad (\text{D41})$$

Combining now the general μ^a model (D36) with the chemical equation of state, Eq. (D26), we get

$$RT \ln \left(\frac{p^a(n^a, \xi, T)}{p} \right) = G_{20}(n^a - n_E^a) + G_{11}(\xi - \xi_E). \quad (\text{D42})$$

From this model-dependent equation and the condition for internal equilibrium (D28), the parameters (G_{20}, G_{11}, G_{02}) can be calculated if two sets of data, namely amounts of water adsorbed and measures for the number of adsorption sites ($(n_1^a, \xi_1), (n_2^a, \xi_2)$) deviating from the equilibria data ((n_E^a, ξ_E)) are known from (spectroscopic) experiments. However, if these data do not belong to internal or conformational equilibrium, Eq. (D28) should not be used. Instead, Eqs. (D34) and (D30) should be taken into account.

Once the parameters (G_{20}, G_{11}, G_{02}) are known, the chemical EOS in its model-dependent form (D42) allows us to calculate either from measured adsorption data (n^a) the conformational parameter (ξ) of the protein in this state or, vice versa, from ξ data the mole number of water adsorbed on the protein's

surface, n^a . Both pieces of information may be useful, as these data are undoubtedly related to the protein’s biological activity in the aqueous solution considered. Naturally, the equations of state of the protein–water system considered so far do not allow us to make any statement on the dynamics of the system and especially its approach to equilibrium ($n_a \rightarrow n_E^a, \xi \rightarrow \xi_E$). For this, it is necessary to apply the basic results of thermodynamics of irreversible processes [D12, D13], which will be the subject of the next section.

A thermodynamic model for hydration processes

Let us again consider the protein–water system sketched in Figure 8; cf. also Figure 5. It consists of the protein itself, the configuration of which is described by an internal variable (ξ) indicating actually the number of adsorption sites for water on its surface, which is related to the number of water molecules n^a actually adsorbed. This “combined thermodynamic system” is surrounded by bulk water at pressure, p , temperature, T , and chemical potential, μ^f , which are assumed to be constant. We assume the protein-adsorbed water system initially to be in a certain equilibrium or a non-equilibrium state (n_0^a, ξ_0) and want to phenomenologically describe its approach to equilibrium, i.e., the exchange process of water adsorbed ($n^a = n^a(t)$) and the conformation adaptation process, $\xi = \xi(T)$. For this, we write down the laws of thermodynamics as:

First Law:

$$dU_m = dQ + h^f dn^a + 0 \tag{D43}$$

Second Law (1) or Gibbs’ equation ($p = \text{const}$):

$$dS_m = \frac{dU_m}{T} - \frac{\mu^a}{T} dn^a + \frac{A}{T} d\xi \tag{D44}$$

Second Law (2) or entropy balance:

$$dS_m = \frac{dQ}{T} + s^f dn^a + dS_{in} \tag{D45}$$

Here U_m indicates the internal energy of the protein–water system, dQ is the differential heat exchanged (quasi-statically) at temperature (T), h^f is the molar enthalpy carried by the bulk water molecules to the adsorbed water phase. The “0” in Eq. (D43) indicates that there is no change in the internal energy of the system if its configuration changes, i.e., if $d\xi \neq 0$.

Similarly, S_m is the entropy of the protein–water system per mole of protein and s^f is the molar entropy of the bulk water. The symbol dS_{in} indicates a differential of the entropy produced during the process. By combining Eqs. (D43)–(D45) we obtain for this quantity, i.e., the entropy production,

$$P_s = \left(\frac{dS_{in}}{dt} \right) = \frac{1}{T}(\mu^f - \mu^a) \dot{n}^a + \frac{A}{T} \dot{\xi} \geq 0. \quad (D46)$$

The first term refers to the mass exchange due to ad- or desorption of water to or from the surface of the protein, and the second term is due to (irreversible) conformational changes of the protein.

The process equations that can be derived according to Eckart and Onsager from Eq. (D46) are in linear approximation [D12, D13]:

$$\dot{n}^a = L_{aa}(\mu^f - \mu^a) + L_{ac}A, \quad (D47)$$

$$\dot{\xi}^a = L_{ca}(\mu^f - \mu^a) + L_{cc}A. \quad (D48)$$

Here the so-called phenomenological coefficients ($L_{aa}(p,T) \dots (L_{cc}(p,T))$) have, according to Eq. (D46), to obey the inequalities

$$L_{aa} > 0, L_{aa}L_{cc} - L_{ac}L_{ca} \geq 0, L_{cc} > 0. \quad (D49)$$

Besides, the Onsager relation ($L_{ac} = L_{ca}$) should hold.

We now restrict ourselves to processes without “cross effects,” i.e., we assume for simplicity’s sake that $L_{ac} = L_{ca} = 0$. Also, we introduce the abbreviations

$$\begin{aligned} \Delta n^a &= n^a(t) - n_E^a \\ \Delta \xi &= \xi(t) - \xi_E, \\ \Delta \mu &= \mu^f(p, t) - \mu^a(t) \end{aligned} \quad (D50)$$

where (n_E^a, ξ_E) indicate the equilibrium values of the amount of water adsorbed on the protein and the configuration parameter of the protein at $(p = \text{const}, T = \text{const}, \mu(p, T) = \text{const})$. In this approximation, the constitutive equations (D47) and (D48) reduce to

$$\Delta \dot{n}^a = L_{aa} \Delta \mu, \quad (D47a)$$

$$\Delta \dot{\xi} = L_{cc} A. \quad (D48a)$$

Inserting these equations into the equations of state (D26) and (D27), we obtain

$$-\frac{1}{L_{aa}} \Delta \dot{n}^a = G_{20} \Delta n^a + G_{11} \Delta \xi, \quad (D51)$$

$$-\frac{1}{L_{cc}} \Delta \dot{\xi} = G_{11} \Delta n^a + G_{02} \Delta \xi. \quad (D52)$$

These are two ordinary differential equations (ODEs) for the time-dependent quantities $n^a(t)$, $\xi(t)$. For given initial conditions ($t = 0$),

$$\begin{aligned} n^a(0) &= n_0^a \\ \xi(0) &= \xi_0, \end{aligned} \quad (D53)$$

and, according to Eqs. (D51) and (D52), also

$$\begin{aligned} \Delta \dot{n}_0^a &= -L_{aa}(G_{20} \Delta n_0^a + G_{11} \Delta \xi_0) \\ \Delta \dot{\xi}_0 &= -L_{cc} G_{02} \Delta \xi_0 + G_{11} \Delta n_0^a, \end{aligned} \quad (D54)$$

the solutions are

$$\Delta n^a(t) = N_1 e^{p_1 t} + N_2 e^{p_2 t}, \quad (D55)$$

$$\Delta \xi(t) = C_1 e^{p_1 t} + C_2 e^{p_2 t}, \quad (D56)$$

with the always real eigenvalues

$$\begin{aligned} 2p_{1,2} &= -(L_{aa} G_{20} + L_{cc} G_{02}) \\ &\pm \left[(L_{aa} G_{20} - L_{cc} G_{02})^2 + L_{aa} L_{cc} G_{11}^2 \right]^{\frac{1}{2}}, \end{aligned} \quad (D57)$$

and the constants (N_1 , N_2 , C_1 , C_2) given by

$$N_i = \frac{1}{p_{i+1} - p_i} (p_{i+1} \Delta n_0^a - \Delta \dot{n}_0^a), \quad i = 1, 2(\text{mod}2), \quad (D58)$$

$$C_i = \frac{1}{p_{i+1} - p_i} (p_{i+1} \Delta \xi_0 - \Delta \xi_0), \quad i = 1, 2(\text{mod} 2). \quad (\text{D59})$$

Therefore, the relations (D55) and (D56) always describe pure or monotonic relaxation processes without any oscillations. Of course, this was to be expected as the adsorption and the conformation process according to Eqs. (D47) and (D48) dynamically were uncoupled or independent from each other. However, if such a coupling exists, an oscillating approach to equilibrium would be possible, leading also to periodically changing biological properties of the protein. Such a behavior also may occur if the environment of the water-coated protein, i.e., the temperature (T) and pressure (p) of the surrounding bulk water, are changing in time. In such a situation, the formalism presented above has to be modified as follows:

We start by again considering the process equations (D47) and (D48), which in principle provide two ODEs for the amount of water adsorbed on the protein $n^a(t)$, and the structure or conformation parameter $\xi(t)$ of the protein. However, the two “thermodynamic forces” occurring in these equations have to be specified in more detail. Especially, their relation to the environmental parameters, namely pressure, p, and temperature, T, must be given explicitly. For the sake of simplicity, we will restrict ourselves to an idealized situation where for example the adsorbate on the protein’s surface can be considered as an “ideal” one; that is, we have for the chemical potential of the sorbate μ^a in any non-equilibrium state at sorbate amount n^a

$$\mu^a(n_1^a, T, \xi) = \mu^f(p, T) + RT \ln \left(\frac{n^a}{n_E^a(p, T, \xi)} \right). \quad (\text{D60})$$

Here $n_E^a(p, T, \xi)$ is the equilibrium value of n^a at (p, T, ξ), referring to the chemical potential of the surrounding fluid $\mu^f(p, T) = \mu_E^a(p, T)$, which is equal to the chemical potential μ_E^a of the adsorbed phase in this case [D10, D11]. For the equilibrium load of the adsorbate, we propose a Langmurian model [D11], i.e.,

$$n_E^a(p, T) = \xi_E(p, T) \cdot \frac{bp}{1 + bp}, \quad (\text{D61})$$

with the inverse of the half-load pressure,

$$b^{-1}(T) = p_a \exp \left(- \frac{Q_a}{R} \left(\frac{1}{T} - \frac{1}{T_a} \right) \right), \quad (\text{D62})$$

and the equilibrium conformation function,

$$\xi_E(p, T) = \Xi(p) \exp \left(- \frac{Q_s}{R} \left(\frac{1}{T} - \frac{1}{T_a} \right) \right), \tag{D63}$$

$$\Xi(p) = \Xi_0 e^{-p/p_s} + \Xi_\infty (1 - e^{-p/p_s}). \tag{D64}$$

That is, the conformation parameter $\xi_E(p, T)$ is assumed to be proportional to the number of adsorption sites available in an arbitrary non-equilibrium or equilibrium state, respectively. Mind that Eq. (D63) implies the limiting relations

$$\lim_{T \rightarrow \infty} \xi_E(p, T) = \Xi(p) e^{Q_s/RT_a}, \quad \lim_{T \rightarrow 0} \xi_E(p, T) = 0, \tag{D63a}$$

the physical consequences of which still have to be checked experimentally.

In Eq. (D62), the quantities p_a, T_a indicate pressure and temperature of a reference state and Q_a is the adsorption enthalpy of the water. Also, p_s in Eq. (D64) is a parameter indicating how fast the protein at increasing pressure ($p \gg p_s$) is changing its low-pressure structure, Ξ_0 , and approaching its high pressure configuration, Ξ_∞ .

Also for the activity, $A(p, T, \xi)$ of the conformation parameter, ξ , we assume a linear driving force model, i.e., a Taylor-series expansion truncated after the first-order term:

$$A(p, T, \xi) = 0 + a(\xi - \xi_E(p, T)) + O(2), \tag{D65}$$

with $\xi_E(p, T)$ given by Eqs. (D63) and (D64) and a being a dynamic parameter to be determined experimentally. Naturally, in any equilibrium state we have $\xi = \xi_E(p, T)$ and $A(p, T, \xi_E) = 0$.

We now are in a position to rewrite the process equations (D47) and (D48) and to clearly separate the dynamic quantities n^a, ξ from the equilibrium parameters μ_E^a, ξ_E of the system:

$$\begin{aligned} \dot{n}^a + L_{aa}RT \ln(n^a) - L_{aca}\xi &= \\ &= L_{aa}RT \ln(n_E^a(p, T)) - L_{aca}\xi_E(p, T) \end{aligned} \tag{D66}$$

$$\begin{aligned} \dot{\xi} + L_{ca}RT \ln(n^a) - L_{cca}\xi &= \\ &= L_{ca}RT \ln(n_E^a(p, T)) - L_{cca}\xi_E(p, T) \end{aligned} \tag{D67}$$

Assuming pressure and temperature to be prescribed functions of time as they may be in a continuously stirred tank reactor (CSTR),

$$p = p(t), T = T(t),$$

Eqs. (D66) and (D67) are two ordinary differential equations for $n^a = n^a(t)$ and $\xi = \xi(t)$, which can be solved numerically in the usual way. Of course, prior to this all the parameters,

$$L_{aa}, L_{ac} = L_{ca}, L_{cc}, p_a, Q_a, a, Q_s, \bar{\Xi}_0, \bar{\Xi}_\infty, p_s, \quad (\text{D68})$$

occurring in these equations have to be known, i.e., determined from selected experiments. In such a situation, it is always worthwhile to rewrite Eqs. (D66) and (D67) in dimensionless variables and to apply Buckingham's theorem from dimensional analysis to restrict to dimensionless combinations of the parameter set (D68). Also, introduction of an appropriate system of orthogonal functions and – first of all – restriction to special isobaric or isothermal situations, would be helpful in order to simplify the analysis and reduce computer time for getting solutions $n^a(t)$, $\xi(t)$ for these equations.

Now it would be worthwhile to apply the foregoing thermodynamic formalism to a real protein–water system and to try to connect analytical results with experimental data. In view of the broad biophysical/biochemical literature available, this seems to be possible in principle [D18, D19]. However, due to restrictions in space and time, we refrain from this but would like to encourage our younger colleagues to consider this for the benefit of biotechnological process development.

List of symbols

A	J/(mol [ξ])	Affinity or activity related to the internal variable (ξ) of the protein–water adsorbate system
f	Pa	Fugacity of the bulk water surrounding the protein–water adsorbate system
G^f	J/mol	Gibbs free enthalpy of the water surrounding the protein–water adsorbate system per mole of protein
G_m	J/mol	Gibbs free energy of a protein–water adsorbate system per mole of protein

H	mol/J	Henry's constant in the non-equilibrium isotherm of the water adsorption process on the protein
$H_E(T)$	Pa^{-1}	Henry's constant in the Freundlich isotherm of water being adsorbed on the surface of a protein
$L_{aa} \dots L_{cc}$	(variable)	Phenomenological coefficients in process equations (D47) and (D48) describing the kinetics of the water adsorption/desorption process to or from the protein and the related conformation process
n	mol/mol	Total mole number of water in the protein–water solution per mole of protein
n^a	mol/mol	Number of moles of water adsorbed on a protein per mole of protein
n_E^a	mol/mol	Number of moles of water adsorbed on a protein per mole of protein in an equilibrium state of the protein–water system
n^f	mol/mol	Number of moles of water in the vicinity of protein per mole of protein
p	Pa	Pressure
p^+	Pa	Pressure of a reference state of the protein–water system
$P_s = (dS_{in}/dt)$	W/K mol	Entropy production during an adsorption or desorption process of water on a protein
Q_a	J/mol	Energy of adsorption of water on the surface of a protein per mole of protein
Q_s	J/mol	Energy of conformation change of a protein due to adsorption of water per mole of protein
S^f	J/mol K	Entropy of the bulk water in the surroundings of the protein–water adsorbate system per mole of protein
s^f	J/K mol	Molar entropy of the bulk water in the surroundings of the protein–water adsorbate system

$S_{in}(Z_0 \rightarrow Z)$	J/K mol	Entropy produced during an adsorption or desorption process of water on a protein starting in a certain state (Z_0) and arriving at state (Z)
S_m	J/mol K	Entropy of a protein–water adsorbate system per mole of protein
T	K	Temperature
U_m	J/mol	Internal energy of a protein–water adsorbate system per mole of protein
V^f	m^3/mol	Volume of the bulk water in the surroundings of the protein–water adsorbate system per mole of protein
V_m	m^3/mol	Volume of a protein–water adsorbate system per mole of protein
μ^a	J/mol	Chemical potential of water molecules being adsorbed on the surface of proteins
μ^f	J/mol	Chemical potential of the bulk water in the vicinity of a protein–water adsorbate system
ξ	variable	Internal variable of a thermodynamic system. Often but not necessarily an extensive variable for which a balance equation and an equation of state can be formulated.
ξ_0	variable	Value of an internal variable in a certain reference state
ξ_E	mol/mol	Number of adsorption sites for water on the surface of a protein per mole of protein in a full or unrestricted equilibrium state

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E. Concluding remarks

The main purpose of this article was to provide an introductory overview of the possibilities thermodynamics offers to describe collective phenomena, i.e., both equilibria states and processes in biofluids, i.e., aqueous or non-aqueous solutions of biochemical molecules like proteins, enzymes, etc. In restricting the presentation to the problems of thermal stability and hydration of proteins, the author is well aware that even in this very limited field of biothermodynamics much is missing in this treatise. Particularly, special phenomena in protein solutions like aggregation [E1, E2] or, more generally, the phase behavior, i.e., precipitation or crystallization of proteins from the liquid solution, have not even been mentioned [E3, E4]. Also, the basic question of interactions

between biomolecules and their consequences with respect to solubility in water, change of phases, or calorimetric effects has not been addressed [E5, E6]. Also, experimental methods necessary to determine the various thermodynamic coefficients, equations of state, or fundamental equations for (G, S, etc.) have not been discussed in detail. Moreover, the background question on how to extend basic thermodynamic concepts to deal adequately with systems of growing complexity in stationary or instationary states far from equilibrium is still open. The basic concept of additional, so-called internal variables used in this article seems to be promising but is still to be debated in the realm of biotechnological experiences. Finally, it seems to be adequate to think about new mathematical methods and their use in real biotechnical systems and processes where normally only very little “hard” information is available, but “experience” – for example that of the beer-brewing colleagues – is decisive. Examples for these new methods, which are already available and indeed are more and more in use in different fields of science and technology, are different kinds of soft computing methods using fuzzy logic, neuro-fuzzy algorithms, neuro-networks, or new types of statistics using results of artificial intelligence research, etc. [E7, E8].

Despite all these deficiencies, it is sincerely hoped that the reader will still find this article to be encouraging and useful for his/her work in many fields of life sciences and biotechnology whenever phenomena occurring not just between a few selected molecules or particles, but between many of them, i.e., phenomena having collective character, have to be described on a macroscopic, phenomenological level. For this purpose, thermodynamics provides a method or even a recipe of how to proceed which is more safe and also more fruitful than many of the other approaches available today [E9].

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