Introduction to the Special Issue: Thermodynamic Aspects of Cryobiology

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Abstract

This brief manuscript introduces the subject of thermodynamics and the papers of the special issue on thermodynamic aspects of cryobiology. Thermodynamic terminology is defined for the non-specialist.

Keywords

terminology, definition, thermodynamics, energy, entropy

Thermodynamics is the study of mathematical relationships arising from physical laws governing energy and entropy. The applicability of those mathematical relationships depends critically on the definitions of the *properties* that appear in the equations; thus, thermodynamics pays particular attention to the precise definition of the properties of matter. Since the mathematical relationships depend on the state and phase of matter, thermodynamics also pays attention to careful descriptions of *states* and *phases* (vapour, liquid, solid, glass [16], etc.). Though many different properties appear in thermodynamics, a very important thermodynamic property is temperature. Since cryobiology is defined by a change in temperature from physiological, it is clear that the fundamentals of thermodynamics are central to any mathematical modeling of cryobiological processes. Furthermore, since many biological processes are suppressed at lowered temperatures, the fate of cells both during and following exposure to low temperatures is often primarily determined by physico-chemical processes (including ice nucleation with the concomitant increase in concentration of solutes in the residual liquid, and osmotic transport across cell membranes) which can be understood using thermodynamics developed for non-biological applications over the past 200 years.

Interest in thermodynamic principles dates back thousands of years. Thermodynamics is usually conceptualized using one of two approaches: the *historical approach* or the *postulatory approach*. The historical approach is based on several laws numbered in the order that they were identified historically. The *First Law of Thermodynamics* recognizes that energy is conserved, thereby providing a variety of equations for the conversion of energy from one form to another (e.g. from potential to kinetic energy). An important concept is *internal energy*. Internal energy is the energy of a system that is not part of either the system's total potential energy with respect to an external reference position or the system's total kinetic energy with respect to an external reference frame. Like all energies, internal energy is only defined with respect to a reference. Internal energy includes the potential and kinetic energies of molecules within the system. When the temperature of a system is changed (without the system's overall potential and kinetic energies changing) it is the internal energy that changes. Internal energy also changes when a system changes phase (e.g. from a solid to a liquid). The *Second Law of Thermodynamics* can be stated many ways and relates generally to the property *entropy*. Entropy also has many definitions but can be understood as being related to the number of choices molecules have for arrangement and distribution of energy. A gas has more possibilities for molecular positions and velocities than a liquid and so a gas has more entropy than a liquid at the same temperature and pressure. The second law of thermodynamics recognizes the tendency for the entropy of an *isolated system* (no exchange of mass, volume or energy with the surroundings) to increase with time, and can be understood to lead to the observations that processes spontaneously occur only in certain directions (e.g. heat flows from a hot body to a cold body). The *Third Law of Thermodynamics* states that the entropy of an ideal crystal at absolute zero (0 degrees Kelvin, *K*) is equal to zero. The *Zeroth Law* recognizes that if a first body is in thermal equilibrium with a thermometer, and a second body is also in thermal equilibrium with the same thermometer, then the two bodies have the same temperature. The zeroth law is so named because, though stated chronologically after the third law, it was realized that it should have logically preceded the other three.

The postulatory approach was developed most notably by J. Willard Gibbs at the end of the 19th century. Rather than stating the foundations of thermodynamics in their historical forms, Gibbs approach was to recast thermodynamics in terms of a small number of postulates needed to mathematically describe the current understanding of thermodynamics. A famous quotation from Gibbs captures his intention, "*One of the principal objects of theoretical research in any department of knowledge is to find the point of view from which the subject appears in its greatest simplicity.*" [9] In 1876 and 1878 J. W. Gibbs published, in two parts, arguably the most influential scientific work of the 19th and 20th centuries, providing the foundation of chemical thermodynamics, phase equilibrium and reaction equilibrium, and describing the role of free energy and chemical potential [10]. I will not describe all the postulates here, but just mention a few important points.

One postulate assumes that internal energy, U, of a simple system is a function only of a specific set of independent variables: entropy, S, volume, V, and the number of moles of each component in a system of r components, N_1, N_2, …, N_r. This function, called *the fundamental relation*, is postulated to have certain mathematical properties about which one can read elsewhere [1],[10]. A *simple system* is defined as a system which follows this postulate and is homogenous throughout at equilibrium. By definition, systems with interfacial, gravitational, magnetic, or other effects not captured in this postulate, are not simple systems. Differentiating the fundamental relation gives

$$dU = \left(\frac{\partial U}{\partial S}\right)_{V,N_1,N_2,\dots,N_r} dS + \left(\frac{\partial U}{\partial V}\right)_{S,N_1,N_2,\dots,N_r} dV + \sum_{j=1}^{r}\left(\frac{\partial U}{\partial N_j}\right)_{S,V,N_1,\dots,N_{i\neq j},\dots,N_r} dN_j \tag{1}$$

The properties U, S, V and N_j are called *extensive properties* because if one adds together two identical systems to form a new composite system, the values of these additive properties for the composite system will be double those of the original systems. By contrast, properties such as temperature are *intensive*, meaning that if one adds together two identical systems, the value of the property will not change. The following equation is a statement of the first law of thermodynamics:

$$dU = TdS - PdV + \sum_{j=1}^{r} \mu_j dN_j \tag{2}$$

Comparing equations (1) and (2) reveals the definitions of the intensive properties *temperature*, T, *pressure*, P, and *chemical potential of the* j^{th} *component*, μ_j.

$$T = \left(\frac{\partial U}{\partial S} \right)_{V, N_1, N_2, \ldots, N_r} \tag{3}$$

$$P = -\left(\frac{\partial U}{\partial V} \right)_{S, N_1, N_2, \ldots, N_r} \tag{4}$$

$$\mu_j = \left(\frac{\partial U}{\partial N_j} \right)_{S, V, N_1, \ldots, N_{i \neq j}, \ldots, N_r} \tag{5}$$

Other important consequences follow from the postulates, such as the *Gibbs phase rule* that asserts that the state of a simple system is completely defined by two independent intensive properties. *Equations of state* are equations that express the relationships between properties for a particular phase but contain less information than the full fundamental relation. Examples of equations of state include relationships between P, V and T, or relationships between μ_j and T, P and $N_1, N_2, \ldots, N_r$. A well known P-V-T relation is the *ideal gas law:*

$$PV = NRT \tag{6}$$

where R is the *universal gas constant*. An example of a relationship between μ_j and T, P and $N_1, N_2, \ldots, N_r$ is the *osmotic virial equation* [6],[14].

An important concept is that of thermodynamic *equilibrium*. A simple system is in equilibrium if there are no spontaneous changes happening with time and all of its intensive thermodynamic properties are homogeneous throughout the system. A *composite system* (not necessarily a simple system but composed entirely of simple systems) is in equilibrium if each of its constituent simple systems is independently in equilibrium and there is no net transport of extensive properties occurring between subsystems. It is a postulate of thermodynamics that an isolated system will be in equilibrium when its entropy is an extremum (entropy is a maximum for a *stable equilibrium state*). It is equivalent to say that an isolated system will be in equilibrium when its internal energy is an extremum at fixed entropy (internal energy, at fixed entropy, would be a minimum for a stable equilibrium state).

Often we are interested in systems which are not isolated, but rather interact with their surroundings in some way. In these cases, the thermodynamic equilibrium of the system is determined by an extremum in the *free energy*. The free energy is the energy of the system free to be minimized once the constraints of the interaction with the surroundings are met. We say that the free energy acts as the *thermodynamic potential* for the system. For example, the *Helmholtz free energy*, *F*, is defined

$$F = U - TS \tag{7}$$

For a constant volume system with its temperature imposed by an external bath with which the system can exchange energy, the Helmholtz free energy will be a minimum at stable equilibrium. For a simple system with both its temperature and pressure held constant by an external reservoir with which the system can exchange both energy and volume, the *Gibbs free energy*, *G*, defined by

$$G = U - TS + PV \tag{8}$$

will be a minimum at stable equilibrium. The property *enthalpy*, H, is defined

$$H = U + PV \tag{9}$$

so that Gibbs free energy may be written

$$G = H - TS \tag{10}$$

The above commonly used equation illustrates that Gibbs free energy has both enthalpic (energetic) and entropic contributions.

Minimizing the free energy is equivalent to maximizing the entropy subject to the constraints imposed. Care must be exercised in determining what function acts as the thermodynamic potential for non-simple systems such as multiphase composite systems exhibiting surface effects, where even for a system able to exchange energy and volume with a surrounding reservoir the system free energy is not the Gibbs free energy, but rather a more complicated function [4],[11]. Cryobiology involves composite systems made up of ice, solution, or vitrified phases in the intracellular and extracellular compartments; even the phospholipid membrane can be treated as a constituent subsystem.

The above description of the foundation of thermodynamics is based on a certain choice of the independent extensive variables but this is not the only possible choice. For example, one can treat a surface phase by postulating that the internal energy depends only on the independent extensive properties entropy, S, area, A, and the number of moles of each component present in the surface phase, N_1, N_2, …, N_r. Following a similar mathematical formalism, one then arrives at *surface thermodynamics* where the *interfacial tension*, γ, is naturally introduced as an intensive parameter

$$\gamma = \left(\frac{\partial U}{\partial A}\right)_{S,N_1,N_2,\ldots,N_r} \tag{11}$$

All of the above is the traditional equilibrium thermodynamics based on the concept of simple systems, or composite systems made up of simple systems, existing at thermodynamic equilibrium. However, in the subject of *nonequilibrium thermodynamics* (also called *irreversible thermodynamics*) fluxes of heat, mass or other extensive properties are related to gradients in intensive properties—and then many of the relationships developed in equilibrium thermodynamics can be used to understand rate processes. For example, heat flux may be assumed to be caused by temperature gradients. In *Fourier's Law of heat conduction*, the heat flux is assumed to be proportional to a temperature gradient, ∇T. In *Onsager linear irreversible thermodynamics*, heat flux is assumed proportional to a gradient in the inverse temperature, $\nabla(1/T)$ [1]. As another example of nonequilibrium thermodynamics, the rate of osmotic transport of water or cryoprotectants across cell membranes can be assumed to be proportional to the difference across the membrane in chemical potential of the transporting species [8]. Diffusion through a bulk and various phenomena associated with the kinetics of ice crystal growth [12] may also be included within nonequilibrium thermodynamics.

The above descriptions of thermodynamics are from the macroscopic viewpoint. That is, the thermodynamic properties, such as temperature, are observable macroscopically and defined for a collection of a macroscopic number of molecules. In fact, all of the preceding formalisms can be applied without knowledge of the existence of molecules. However, thermodynamics is completely consistent with the existence of molecules. If one has detailed molecular knowledge (either quantum mechanical or classical mechanical) that knowledge can be averaged for an *ensemble* (a carefully specified collection of systems) to arrive at macroscopic thermodynamic knowledge. The study of thermodynamics making explicit use of molecular information is called *statistical thermodynamics*. *Statistical Rate Theory* is a theory of nonequilibrium thermodynamics with a statistical foundation resulting in a nonlinear relationship between rates and driving forces [5],[7].

The papers in this special thermodynamics issue cover several different aspects of thermodynamics relevant to cryobiology. The paper by Wowk [16] describes the thermodynamic aspects of vitrification (the formation of a glass from a liquid solution). The contribution from Preciado and Rubinsky [13], details their work on isochoric preservation, in which the independent intensive thermodynamic variables in cryopreservation are changed from temperature and pressure (T and P) to temperature and specific volume (T and v). The paper by Prickett, Elliott and McGann [14], reviews their work on the osmotic virial equation as a solution theory for cryobiology. Karlsson [12] describes the implementation of his model of intracellular ice nucleation, and demonstrates that the thermodynamic and kinetic behavior of intracellular ice formation (IIF) predicted by his model is more realistic than corresponding predictions of simpler IIF models. Rossi, Tanaka, Shimada and Rabin [15] describe bioheat transfer computer simulations for cryosurgical planning. The paper by Devireddy [2] presents an overview of the statistical thermodynamic treatment of phospholipid membranes and describes the lipid membrane interactions with a cryoprotective agent, dimethyl sulfoxide. The review by Dias, Ala-

Nissila, Wong-ekkabut, Vattulainen, and Karttunen [3] explains their previous computations giving a detailed molecular and thermodynamic understanding of the hydrophobic effect driving the cold denaturation of proteins.

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