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Review

Foundations of modeling in cryobiology—I: Concentration, Gibbs energy, and chemical potential relationships ☆

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ABSTRACT

Mathematical modeling plays an enormously important role in understanding the behavior of cells, tissues, and organs undergoing cryopreservation. Uses of these models range from explanation of phenomena, exploration of potential theories of damage or success, development of equipment, and refinement of optimal cryopreservation/cryoablation strategies. Over the last half century there has been a considerable amount of work in bio-heat and mass-transport, and these models and theories have been readily and repeatedly applied to cryobiology with much success. However, there are significant gaps between experimental and theoretical results that suggest missing links in models. One source for these potential gaps is that cryobiology is at the intersection of several very challenging aspects of transport theory: it couples multi-component, moving boundary, multiphase solutions that interact through a semipermeable elastic membrane with multicomponent solutions in a second time-varying domain, during a two-hundred Kelvin temperature change with multi-molar concentration gradients and multi-atmosphere pressure changes. In order to better identify potential sources of error, and to point to future directions in modeling and experimental research, we present a three part series to build from first principles a theory of coupled heat and mass transport in cryobiological systems accounting for all of these effects. The hope of this series is that by presenting and justifying all steps, conclusions may be made about the importance of key assumptions, perhaps pointing to areas of future research or model development, but importantly, lending weight to standard simplification arguments that are often made in heat and mass transport. In this first part, we review concentration variable relationships, their impact on choices for Gibbs energy models, and their impact on chemical potentials.

1. Introduction

The mechanical and physical chemical nature of cells cooling to and warming from cryogenic temperatures has continually provided a well-understood theoretical mechanism for defining models that capture, at least, the key elements of the process. Mazur's paper deriving a model of the mass transport of water from a cell during cooling [85] and Mazur, Leibo and Chu's explanation of the correlation of cryopreservation success with different cooling rates [87] suggested that coupling standard physical chemistry with classical membrane transport physiology could result in a very useful predictive model [85].

Assuming a constant cooling rate, $T'(t) = B$, Mazur used a linear ideal transport model in conjunction with an ideal model of vapor pressure of water in an ideal dilute solution to derive the differential equation

$$Te^{b(T_0-T)}\frac{d^2V}{dT^2} - \left[(bT+1)e^{b(T_0-T)} - \frac{ARk_0n_2}{B(V+n_2v_1^0)}\cdot\frac{T^2}{V}\right]\frac{dV}{dT} = \frac{LAk_0}{Bv_1^0}, \quad (1)$$

where V is volume, T temperature, A surface area, L latent heat of fusion, k permeability constant, b a temperature dependence parameter for permeability, n_2 is moles of nonpermeating solutes, v_1^0 is a molar volume of solutes, and subscript 0 indicates a value at a reference temperature. This model makes several key

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assumptions admitted in the original manuscript, including the assumption of ideal dilute solutions when in fact solutions cooled to even −5 °C in the presence of ice are fairly non-ideal and non-dilute environments.

This assumption aside, it was (and still is) particularly useful as a theoretical tool, serving as a foundation for the classical "two factor hypothesis" [87] that corroborated experimental results showing that there was a quasi-equilibrium optimal cooling rate, and even how to determine it [86]. Briefly, the two factor hypothesis states that cell death during non-ultrarapid cooling (i.e. cooling rates too low to avoid significant extracellular crystallization) are attributed to two competing factors. The first is that cells cooled too quickly do not have time to sufficiently dehydrate, causing intracellular ice to form, killing the cell. The second is that cells cooled too slowly cause injury due to extended exposures to high solute concentrations. Therefore the "optimal" cooling protocol is the fastest that avoids intracellular ice formation. With over 100 citations between 2008 and 2013, the two-factor hypothesis is still the predominant theory of cooling induced cryoinjury,[1] though recent work by Seki et al. on ultrarapid (e.g. 10^6 K/s) warming of samples has opened this hypothesis to some questions [110]. In particular, Seki et al. demonstrate survival to be independent of the cooling rate. However, the mechanisms to avoid damage from the two different approaches (quasi-equilibrium cooling or ultrarapid warming) are different and not mutually exclusive. For likely different reasons, it has been demonstrated that sperm are relatively incapable of intracellular ice formation [91,92], suggesting that the "two-factor" hypothesis is not applicable at least in the case of sperm.

The success of this model, and more thermodynamically accurate models to follow (e.g. [71,72,112] and others), defined cryobiology as a science where much could be gained even by relatively simple models making broadly unrealistic assumptions. In fact as cryobiology has progressed as a discipline, various researchers have made use of models to determine optimal cooling and/or warming profiles [16,51,81,102,103,117,122], optimal pre- and post- cooling processing protocols [11,12,37,39,69,93,108], intracellular ice formation kinetics [3,45,46,119,125], ice damage in and optimal cryopreservation of tissues [1,2,23,31,99,111,114,126,127], among others.

While Mazur's work laid the foundation for modeling success in cryobiology, another foundation of cryosuccess is a chemical one: cryopreservation nearly universally requires the presence of cryoprotective chemicals, known as cryoprotective agents or CPAs. The most common CPAs are from a class of solutes that include many small-molecular polyols such as glycerol, ethylene glycol (ethane-1,2-diol), and propylene glycol (propane-1,2-diol), in addition to dimethyl sulfoxide (Me_2SO). These CPAs share the common feature that they are membrane permeable, and their positive effect on cryosurvival is attributed to several factors, including that they mostly benignly reduce the relative salt concentration at any given osmolality and they increase extra- and intra-cellular viscosity encouraging glass formation devoid of potentially deleterious intracellular ice.

While solute–solvent membrane transport models have been used for a very long time in physiologic or near-physiologic conditions [42,64,65,83], and while it was understood that CPAs were critical for cell survival by the early 1950s, CPAs are left unaccounted in Eq. (1) and many other follow up manuscripts [45,46,119]. There are reasonable arguments for this omission: for example, low temperature reductions in already relatively low CPA membrane permeability suggest that there is little CPA transport in many cryoprotocols. However, this is not always the case, and sub-zero CPA transport has been modeled and utilized in several novel applications [81,88], though most of the underlying assumptions, including an ideal-dilute transmembrane flux model with ideal-dilute osmotic pressure models, have usually been retained.

With the exception of a few individual manuscripts, the omission of CPA transport is likely due to the difficulty of determining the parameters for non-ideal solution models, and the Occam's razor question of the utility of complicated modeling paradigms versus the speed and simplicity of simple paradigms, especially given the success of Mazur's simple water-transport-only model. In the governing theory of membrane transport in cells and tissues it is nearly universal that fluxes are considered proportional to forces (c.f. [32] for a review and discussion). This is carried out in both "standard" cellular mass transport models used in cryobiology, namely the so called "two parameter" [42,52] and "Kedem–Katchalsky" models [54,55] (c.f. [56] for a review and comparison). In short, both suppose that membrane fluxes are linearly dependent on transmembrane chemical potential gradients. Many non-ideal models of transport exist, however. For example, Elliott et al. propose using a model derived from Statistical Rate Theory that essentially accounts for the likelihood that molecules cross the membrane barrier [28], and the utilization of this transport model with nonideal nondilute models of chemical potential should yield a fully nonideal nondilute modeling paradigm. In fact, Benson showed mathematically that the local stability of the equilibria, and thus the behavior near equilibrium, of this new system is equivalent to the stability for the linear Fickian paradigm [10].

Kleinhans's review of transport models [56] argues against using the Kedem and Ketchalsky paradigm in favor of the so called two parameter or 2P model:

$$\begin{aligned} \frac{dV_w}{dt} &= -P_w A(\pi^e - \pi^i), \\ \frac{dN_s}{dt} &= P_s A(M_s^e - M_s^i), \end{aligned} \tag{2}$$

where V_w and N_s are the intracellular water volume and moles of solutes, respectively, P_w and P_s are water and solute permeabilities, A is the (usually assumed constant) cell surface area, π is the solution osmolality, and M_s is the molarity of the solute quantified by N_s. The typical (dilute) assumption addressed by Kleinhans is that π is approximated by the sum of the "nonpermeating" and "permeating solute" molalities $(m_s + m_n)$ and, intracellularly, these are essentially equivalent to $m_s^i + m_n^i = (N_s + N_n)/V_w$, where the permeating solute quantified by N_s is the CPA, and N_n is a lumped term describing the moles of all nonpermeating solutes (e.g. $dN_n/dt \approx 0$). This model, then, assumes that ions are membrane impermeable, or at least relatively so.

While rigorous treatment of nonideality in transmembrane water transport in electrolyte solutions for cryobiological application was introduced four decades ago by Levin et al. [75], only fairly recently has this nonideality problem been addressed in the context of nonelectrolyte solutions and transport by some of the work of both Kleinhans and Mazur [57] and, on a more substantial thermodynamic basis, Elliott and collaborators [30,32,96–98]. They have shown that accurate models for complicated multicomponent non-dilute and non-ideal solutions can be obtained by using information from standard binary phase diagrams. In short, both approaches assume that osmolality $\pi := \pi(m_s, m_n)$ is a polynomial in intra- or extracellular molality, whose coefficients may be determined by the binary phase diagrams of the components of the mixture. Essentially, the chemical potential may be expanded as a Taylor series in constituent mole fraction or molalities where the Taylor coefficients have a particular form. In fact, Ross-Rodriguez et al. [104] utilized the new polynomial models to explore the impacts of the idealilty assumption

[1] Ref. [87] has more than 100 citations between 2008 and 2013 alone. (Web Of Knowledge Search: Doi: http://dx.doi.org/10.1016/0014-4827(72)90303-5. Refined by: Publication Years = (2008 OR 2009 OR 2010 OR 2011 OR 2012 OR 2013).

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by comparing an ideal and a nonideal cytoplasmic solution model at equilibrium in subzero temperatures to demonstrate large differences in equilibrium volume predictions between ideal solution models and non-idea solution models in several cell types.

There are non-dilute models for osmolality, and non-dilute improvements to the solute transport term in system (2) may also be derived (see e.g. [10,32]). However, these models are still based on Fickian (linear) constitutive transport models, and may be inaccurate in the cases of large osmotic or chemical potential gradients. The implications of these more thermodynamically appropriate models on cellular state during cryoprotocols have been explored recently by Weng et al. [121]. They compare ideal-dilute approximations to non-ideal, non-dilute models of chemical potential and demonstrate differences in predicted responses to standard linear cooling (and ostensibly warming) protocols. While they recognize the potential for error using a dilute transport theory (essentially Fick's law), they do not explore this phenomena. Importantly, it has not been conclusively shown that the utilization of these more accurate and appropriate models of osmolality offers improved prediction of cryosurvival.

Curiously in the cryobiological literature, little modeling attention is paid to ionic transport, which is nearly universally assumed to be non-existent or to have minimal if any impact on the cellular state as pertains to ice formation. In fact, while Prickett et al. address osmolality in the presence of electrolytes [98], they present a model assuming impermeability of ionic solutes as an alternative definition of the Boyle Van't Hoff equation, or to be more precise, they present it as a model that behaves as the Boyle Van't Hoff equation does in non-ideal, non-dilute solutions [96]. Levin et al. have a series of papers addressing solute polarization in the presence of electrolytes, but assume no transmembrane ionic flux [77]. There is a vast body of literature on ionic transport in a wide variety of settings (though to our knowledge not in the cryopreservation setting—see Mori et al. [89,90] for an elegant treatment of ionic and nonionic solute and solvent transport) but transport of ions in molar quantities that affect intracellular ice formation (e.g. that change the intracellular state enough to affect optimal cooling and warming rates) seem to occur on the timescale of hours at room temperature (see, e.g. [63]). Because the vast majority of superzero cryoprotocols occur on the order of minutes, and even slow cooling protocols occur on timescales of about an hour, ionic transport seems to have been largely neglected in the cryobiology literature. In fact, Levin et al. present an argument suggesting that the ratio of membrane permeabilities of salt and of water, P_{salt}/P_w, is on the order of 10^{-8} [77]. These comments aside, this may be an area worth exploring for potential sources of cryopreservation related damage.

An assumption addressed only occasionally in cryobiological modeling is that of perfect extracellular stirring. Mazur's model above and modern nonideal models such as the one proposed and examined by Weng et al. [121], for example, assume that there is no solute polarization or other chemical potential gradient generated by the sequestration of water into the incoming ice front. Models addressing concentration gradients generated by ice fronts have been proposed (see for example [15]), but these are the exception rather than the rule. At the cell–media interface, there is a large body of literature on so-called unstirred layers in the physiology literature (see, e.g. [7] for review), but with the exception of some of the work by Levin et al. [70,72–74,76,77,101] these are not usually accounted for in the cryobiological literature. This may be due to the fact these unstirred layers do not dramatically affect the nature of the transport model, as the usual treatment is to consider the layers to be fixed in width, and thus modeled as membranes in series which correspond to model (2) with P_s and P_w replaced with phenomenological permeability coefficients accounting for the layers (see, e.g. Levin et al. [74] or Barry and Diamond [7]). This may also be due to the relative kinetics of transmembrane vs intracellular vs extracellular transport, a fact addressed by Levin et al. [77], who found that at cooling rates below 100 K/min, there was negligible concentration polarization. This rate is higher than the great majority of quasi-equilibrium cooling rates used in modern cryobiological practice. Note, additionally, that Levin et al. treat solutions with only water and nonpermeating salts.

To apply transport models towards protocol development, however, the problem with this assumption even in the absence of other extracellular gradients generated, say, by ice fronts or changing CPA concentrations, is that cells are often cryopreserved in quite different environments than those where their permeability measurements are made. To wit, the Coulter counter has become a workhorse for permeability estimation (see [4,9,34,35,38,79,80,82,124,129]) and these measurements are made after cells are rapidly injected into a well stirred environment where cells are drawn through a small aperture. This is in contrast with the cryopreservation of cells in straws or tubes where, unless a flow is generated from temperature gradients, concentration gradients and/or ice fronts, the fluid is motionless. There is a considerable literature on the effects of stirring velocity on solute polarization at semipermeable membranes (see [1,2,7,62,111]). Therefore, care should probably be taken in applying results from one experimental regime to another.

Related to this is that the movement and geometries of the free boundaries of the solution/ice and intra/extracellular interfaces are affected by local concentrations. If solutes are excluded from ice, then at the local solution/ice interface, the melting point is depressed further than in the bulk media. This has the effect of slowing the ice-front, making the impingement of ice towards the cell dependent on extracellular solute transport, governed by the advection–diffusion equation examined both experimentally and theoretically in general systems [21,33,53,59–61,94], and theoretically [43] and experimentally [40] in the case of the cellular response to an advancing ice front. These quantities will also be affected by local temperature effects such as the heat of fusion at the solution/ice interface, generating extracellular thermal gradients that are rarely accounted for, at least in Mazur's model and other models consisting of only ordinary differential equations (such as [107,121]). These quantities will be affected by the local pressure generated by impinging ice fronts in solidifying domains, effects that have been occasionally explored (see, e.g. [18,27]), but not coupled with other potential sources of deviation from the "standard" models. Moreover, these pressure effects may point to sources of "solution damage" , one of the "factors" of the "two factor hypothesis" discussed above, as high pressure has been shown to be damaging to cells [130], but can be a tool to suppress ice formation and increase survival [95,105,106,115].

Up to now we have found many areas of potential discrepancies in the "standard" model used in cryobiological modeling, and even have pointed out several effects that are rarely accounted for in the cryobiological literature. However, the use of models in cryobiology is to assist researchers and clinicians in developing optimal cryoprotocols. Therefore, the need for complicated models involving complicated nonideal/nondilute transport equations, electrochemical differential equations accounting for ionic fluxes, solute polarization due to impinging ice fronts, free cellular boundaries and the like is reflected in their ability to predict "more optimal" protocols than the standard models. For example, though we have discussed complicated nonideal nondilute models of mass transport, their use in ideal-dilute situations is not needed, and, as pointed out in Kleinhans's review [56], add extra parameters that cloud the key issues, and may in fact add unneeded uncertainty to conclusions. Therefore, the overarching question is: what effects are important to cryobiologists? When is it sufficient to use ideal-dilute models? When is it okay to deal with the considerably

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easier equations generated by the assumption of perfect extracellular stirring? When must we account for solute polarization? How should we even approach putting these models together?

Therefore, in this three part series, we propose to build a model from fairly fundamental thermodynamic principles to account for a very wide range of phenomena. Namely, we present the double free-boundary problem of a cell in a multisolute solution (of which the "standard" cryo-system of nonpermeating solute–permeating solute–water such as salt–CPA–water is a member) undergoing cooling where an ice front is approaching. We account for the temperature, concentration, and pressure of the system as a function of space and time, deriving the system in generality, and then progressively restricting to simpler and simpler cases, discussing the effects of these simplifications. We end with a numerical exploration of a one-dimensional (radially symmetric) system with many simplifications that still point to differential cell responses compared to standard perfect stirred models.

This approach is similar to the one used by Batycky et al. who present a cryobiological model beginning with concentrations and a transport theorem, and derive an equation for transport in a spherical cell with moving boundary that includes spatial variation within the cell [8]. Their treatment does not address extracellular spatial gradients in concentration, is isothermal with no external solidification, and does not account for solidification induced pressure gradients. Their treatment also does not start with the fundamental quantities of Gibbs energy or deal with non-ideal solutions.

This is not to say that the thermodynamic energy quantity is ignored in the cryobiological literature. A few (non-exhaustive) examples include the recent work of Elliott et al. [30,32], who begin with choices of a Gibbs energy. Angell and Senapati [6] and Rasmussen and MacKenzie [100] use free energy to describe the phase behavior of solutions under various assumptions. Turov et al. use free energy to quantify bound intracellular water [120]. Devireddy provides a detailed and careful thermodynamic treatment of membranes in the context of transport through bilayers and pores [22].

A more comprehensive review of all physical, mechanical, and chemical effects can be found in Zhmakin [128]. In Zhmakin's excellent and thorough review, he briefly addresses key considerations that we do not, including vitrification, viscosity, nucleation, variation in osmotically active intracellular regions, thermal expansion, and elastic effects of the cell and membrane. While vitrification plays a critical role in cryopreservation, the purpose of our model is to understand the non-vitrification regime of cryopreservation akin to Mazur's model above and its well established inverted "U" survival curves. These other effects may be incorporated into our models with some effort, and present a challenge primarily in the determination of key descriptive parameters of temperature, concentration, pressure, and other dependence. Here we assume the absence of intracellular ice, though our models should facilitate the improved prediction of the probability of intracellular ice formation based on the models of Toner [119] or Karlsson et al. [47]. Another critical concept addressed by Zhmakin is the entrapment versus rejection of cells by an incoming ice front that supports theories of directional solidification. While this is of considerable interest (see, e.g. Hubel et al. [41], Elliott and Peppin [29], or Sobolev [113]), cells will be either "vitrified" or entrapped by ice at some stage of the process, therefore, we choose to start with the cells entrapped.

To start we must define several key quantities:

1. The governing equation for the energy of our system. In this case we define the Gibbs energy and explore the effects of the choice of variables on this energy.
2. Transport equations for both energy (defining heat as a function of time and space) and mass (defining concentration as a function of time and space).
3. Boundary conditions for these governing equations at the solution/ice and cellular membrane interfaces.

We note that each of these quantities has a different natural form depending on geometry and quantities modeled. To wit, it is very natural to work experimentally in terms of molality, yet chemically and thermodynamically, mole-fraction or molarity are more amenable to analysis, as we will see below. Finally—notation is always a challenge when combining multiple modeling paradigms (e.g. heat, pressure, concentrations, phase transformation, etc.) and we have done our best to follow the best practices recommended by the International Union of Pure and Applied Chemistry (IUPAC) [17].

2. Preliminary definitions and composition variables

For cryobiological applications and for other multicomponent diffusion processes, the relative quantities or composition of the chemical constituents involved must be described and/or expressed quantitatively by some appropriate measure (e.g. mole fraction, mass fraction, molarity, molality, etc.). The choice of which compositional measure to use is often dictated by the particular context; a quantity that is convenient for use in a theoretical context, for example, may not be suitable for use in experimental context where actual chemicals are being measured and mixed into solution. As a result, the comparison of different papers, experiments and/or computations often requires the conversion from one measure to another (for example, see the discussion in Appendix A of Kleinhans and Mazur [57]). With this in mind, we find it useful to begin with a review of some of these composition variables and the relationships between them. Note that we have been careful to avoid the term concentration which for us has a specific definition, and have chosen to initially use the term composition to describe the relative quantities of components. Our discussion draws from related ones that can be found in Bird, Stewart and Lightfoot [13], Andersson and Ågren [5], Dantzig et al. [19] and Sekerka [109]. The first part of this discussion, in particular, follows closely the work of Dantzig et al. with the addition here of thermal effects.

Consider a mixture of n components occupying a volume V at temperature T and pressure P. Define N_k to be the number of moles[2] of substance k in the volume, where $k = 1, \ldots, n$. The first composition variable[3] we define is the **mole fraction** x_k of substance k

$$x_k := \frac{N_k}{\sum_{i=1}^{n} N_i}. \tag{3}$$

In general V depends on T, P and N_k. This leads to the differential relationship[4]

$$dV = \left(\frac{\partial V}{\partial T}\right)_{P,N_k} dT + \left(\frac{\partial V}{\partial P}\right)_{T,N_k} dP + \sum_{k=1}^{n} \left(\frac{\partial V}{\partial N_k}\right)_{P,T,N_{j\neq k}} dN_k, \tag{4}$$

$$= \alpha V dT - \beta V dP + \sum_{k=1}^{n} \overline{V}_k dN_k, \tag{5}$$

[2] Recall that a mole is equal to the amount of a substance that contains Avagadro's number ($N_A = 6.02217 \times 10^{23}$) of molecules. Avagadro's number is the number molecules in 12 grams of Carbon-12. The number of moles is the number of molecules of a substance divided by N_A. The molecular mass of a substance in Atomic Mass Units is equal to the mass in grams of one mole of the substance.

[3] We note that Sekerka [109] distinguishes between *concentration* ('…"stuff" per unit volume…') and *composition* which is what is measured by x_k.

[4] Note that we have adopted the practice of explicitly indicating what variables are held constant in the partial differentiation.

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where we define α to be the isobaric thermal expansion coefficient, β the isothermal compressibility and $\overline{V}_k$ the partial molar volume of component k given by

$$\alpha = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_{P,N_k}, \quad \beta = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_{T,N_k}, \quad \overline{V}_k = \left(\frac{\partial V}{\partial N_k}\right)_{P,T,N_{j\neq k}}, \tag{6}$$

where, in general, these quantities are functions of T, P and N_k. It is also true that the volume, an extensive quantity, can be expressed as

$$V = \sum_{k=1}^{n} \overline{V}_k N_k. \tag{7}$$

This is the so-called Euler equation.[5]

As pointed out by Dantzig et al. [19] this result, along with Eq. (4) leads to the condition

$$0 = \alpha V dT - \beta V dP - \sum_{k=1}^{n} N_k d\overline{V}_k. \tag{10}$$

This result implies that there are certain thermodynamic consequences regarding assumptions of constant temperature, pressure and/or partial molar volumes. One further definition at this point is the molar volume of a phase (the volume occupied by one mole of the substance)

$$V_{\mathrm{M}} := \frac{V}{\sum_{i=1}^{n} N_i} = \frac{\sum_{k=1}^{n} \overline{V}_k N_k}{\sum_{i=1}^{n} N_i} = \sum_{k=1}^{n} x_k \overline{V}_k. \tag{11}$$

A second concentration variable (moles of substance k per unit volume of mixture), referred to by Dantzig et al. as **the concentration** but also known as **molarity**, or **molar concentration**, is defined by

$$c_k := \frac{N_k}{V} = \frac{x_k}{V_{\mathrm{M}}}. \tag{12}$$

A concentration of one 'molar' (also sometimes denoted 1 M) is one mol per liter. These definitions imply that

$$\sum_{k=1}^{n} x_k = 1, \quad \sum_{k=1}^{n} c_k = \frac{1}{V_{\mathrm{M}}}, \quad \sum_{k=1}^{n} c_k \overline{V}_k = 1. \tag{13}$$

One further thermodynamic relationship follows from dividing Eq. (10) by V and differentiating the last equation in display (13) to get $\sum_{k=1}^{n}(c_k d\overline{V}_k + \overline{V}_k dc_k) = 0$, yielding

$$0 = \alpha dT - \beta dP + \sum_{k=1}^{n} \overline{V}_k dc_k. \tag{14}$$

A third composition variable is the **molality** of solute k, denoted by m_k. The molality of solute k is the number of moles of solute k divided by the mass (in kilograms) of solvent. Note that in contrast to molarity, which is in general temperature and pressure dependent since it is a volume-based quantity, the molality, being mass-based, is independent of temperature and pressure. One 'molal' (also sometimes denoted 1 m) is one mol per kilogram. If we denote N_1 to be the number of moles of the solvent (e.g. water) and M_1 to be the molecular mass (mass of one mole) of the solvent then the product $N_1 M_1$ is the total mass of the solvent. Therefore, the molality m_k is defined by

$$m_k := \frac{N_k}{M_1 N_1}, \quad \text{for } k = 2, \ldots, n. \tag{15}$$

Also, the molality can be expressed relative to the mole fraction by

$$m_k = \frac{x_k}{M_1 x_1}, \quad \text{for } k = 2, \ldots, n, \tag{16}$$

from which we find that

$$\sum_{i=2}^{n} m_i = \frac{1}{M_1 x_1}(1 - x_1). \tag{17}$$

It follows that

$$x_1 = \left(1 + M_1 \sum_{i=2}^{n} m_i\right)^{-1}, \tag{18}$$

and

$$x_k = \frac{M_1 m_k}{1 + M_1 \sum_{i=2}^{n} m_i}, \quad \text{for } k = 2, \ldots, n. \tag{19}$$

Also note that the molality can be expressed relative to the molarity by

$$m_k = \frac{c_k}{M_1 c_1}, \quad \text{for } k = 2, \ldots, n. \tag{20}$$

The quantity $M_1 c_1$ is the mass concentration of the solvent. The molarity can be obtained from the molality using the expressions

$$c_1 = V_{\mathrm{M}}^{-1}\left(1 + M_1 \sum_{i=2}^{n} m_i\right)^{-1}, \tag{21}$$

and

$$c_k = \frac{M_1 m_k}{V_{\mathrm{M}}\left(1 + M_1 \sum_{i=2}^{n} m_i\right)}, \text{for } k = 2, \ldots, n. \tag{22}$$

The **mass concentration** of species k is the mass of species k (N_k times the molecular mass M_k) per unit volume of solution:

$$\rho_k := \frac{N_k M_k}{V} = c_k M_k = \frac{x_k M_k}{V_{\mathrm{M}}}. \tag{23}$$

The **mass fraction** of species k, defined as the mass of species k divided by the mass of the solution, is

$$\omega_k := \frac{N_k M_k}{\sum_{i=1}^{n} N_i M_i} = \frac{\rho_k}{\rho}, \tag{24}$$

where

$$\rho := \sum_{i=1}^{n} \rho_i, \tag{25}$$

is the mass density of the solution. Note that

$$\sum_{i=1}^{n} \omega_i = 1. \tag{26}$$

Other composition variables (e.g. weight percent, etc.) can be defined and we refer the reader to the classic textbook by Bird, Stewart and Lightfoot [13]. Other discussions prominent in cryobiology can also be found. For example, Elmoazzen et al. [32] discuss the concentration variables **osmolarity** ["number of moles of solute, per liter of solution, of an ideal dilute solute that would be needed to produce the same osmotic activity as a particular concentration of a nondilute solute"], and **osmolality** ["the number of

[5] Volume is a homogeneous function of degree one of the extensive variables N_k. That is,

$$V(T, P, \lambda N_1, \lambda N_2, \ldots, \lambda N_n) = \lambda V(T, P, N_1, N_2, \ldots, N_n), \tag{8}$$

where λ is an arbitrary constant. Differentiating this expression with respect to λ while holding temperature and pressure fixed, and then setting $\lambda = 1$ gives the Euler equation [14,19,109]

$$\sum_{k=1}^{n}\left(\frac{\partial V}{\partial N_k}\right)_{T,P,N_{j\neq k}} N_k = V. \tag{9}$$

moles of an ideal, dilute solute, per kilogram of solvent, which would be needed to produce the same osmotic activity as a particular concentration of a nondilute solute"].

The composition variables x_k, c_k, m_k, ρ_k and ω_k defined above can be viewed in the context of a finite collection of particles occupying some volume in space. However, within such a volume these definitions do not address spatial locations of individual particles. For example, the determination of N_k in the definition of mole fraction in Eq. (3) requires knowledge of the number of moles of substance k within the volume but not the specific location of each particle within the volume. For the applications of interest here, spatial variation of composition variables will be of critical importance. Therefore, we shall interpret the above composition definitions as point-wise definitions in a typical continuum approach (e.g. see Leal [68]). That is, we associate with each mathematical point in some macroscale system of interest (e.g. a cell, a tissue, and/or the surrounding cryo-fluid) a material parcel that is assumed to contain many particles and for which the composition variables can be defined. The typical length scale associated with the macroscale system is much larger than the length scale of the parcel, whose length scale in turn is much larger than the molecular scale. In this way, the composition variables and accompanying relationships defined above apply point-wise in the macroscale and therefore take on spatial (and temporal) dependence.

We note that this continuum approach takes advantage of the thermodynamic principal that the behavior of a system may be approximated by the local average of its constituents' behavior due to the huge number of molecules in the system. However, continuum approaches must be differentiated from approaches where individual molecules are followed as in the case of molecular dynamics simulations or the variation in the actions of individual molecules are allowed as in the case of stochastic diffusion modeling. While these methods are extremely powerful in specific contexts, their usefulness in the very large scale of cells is questionable. Extremely large molecular dynamics simulations requiring enormous computational resources may follow the actions of 10^9 individual molecules. The number of molecules in a single cell of radius 5 μm is on the order of 10^{13}, and the number of molecules in a single cell plus the surrounding 5 μm is on the order of 10^{14}, but by the thermodynamic averaging of continuum modeling, these composition quantities can be calculated accurately with minimal computational power.

3. The motivation: species transport equations

Because we wish to model transport of mass and energy, we begin with a fundamental equation of conservation, the transport equation. Suppose that in an arbitrary time-dependent domain $\Omega(t)$ in space with boundary $\partial\Omega(t)$ we have a quantity of stuff (energy, moles of solute, etc.) with density ψ, which is a function of space and time $\psi = \psi(\mathbf{x}, t)$. Then, a fundamental physical balance, assuming no sources/sinks that add or remove stuff (e.g. no reactions, phase transformation, etc.) within $\Omega(t)$, is

$$\frac{d}{dt}\int_{\Omega(t)} \psi \, dV = -\int_{\partial\Omega(t)} \mathbf{J}\cdot\hat{\mathbf{n}}\, dA,$$

where $\mathbf{J}$ is a total flux of stuff (e.g. convective, diffusive, etc.) across the surface and $\hat{\mathbf{n}}$ is the outward unit normal vector to the surface. It is important to point out that, owing to the continuum approximation, the flux $\mathbf{J}$ includes both average convective transport associated with a macroscale velocity $\boldsymbol{u}$ as well as a molecular contribution such as Fickian diffusion or conductive heat transport (e.g. see Leal [68]). This equation states that in the absence of reactions, etc. the change in the amount of stuff in $\Omega(t)$ is equal to its flux across the domain boundary $\partial\Omega(t)$. The Reynolds transport theorem (sometimes referred to as an extension of Leibnitz Formula [13,68]) implies that

$$\int_{\Omega(t)} \frac{\partial\psi}{\partial t}\, dV + \int_{\partial\Omega(t)} \psi \boldsymbol{u}_b\cdot\hat{\mathbf{n}}\, dA = -\int_{\partial\Omega(t)} \mathbf{J}\cdot\hat{\mathbf{n}}\, dA,$$

where $\boldsymbol{u}_b$ is the velocity of the boundary $\partial\Omega(t)$. Note that physical balances are often expressed in the context of either a 'material' surface that moves with the normal velocity of the material particles at the boundary (so $\boldsymbol{u}_b\cdot\hat{\mathbf{n}} = \boldsymbol{u}\cdot\hat{\mathbf{n}}$) or for a domain that is fixed in time (so $\boldsymbol{u}_b = 0$) and the corresponding flux $\mathbf{J}$ must reflect this choice. Stokes's (Divergence) theorem yields

$$\int_{\Omega(t)} \frac{\partial\psi}{\partial t} dV + \int_{\Omega(t)} \mathrm{div}(\psi\boldsymbol{u}_b) dV = -\int_{\Omega(t)} \mathrm{div}\mathbf{J}\, dV.$$

Because $\Omega(t)$ was arbitrary, this integral identity holds for all regions, and we have the generic transport equation

$$\frac{\partial\psi}{\partial t} + \mathrm{div}(\psi\boldsymbol{u}_b) = -\mathrm{div}\,\mathbf{J}.$$

To fix ideas and motivate the discussion in the sections that follow we consider the transport of a chemical species (e.g. CPA or salt in the intra- or extra-cellular region) measured by concentration c_k, for a scenario in which there is no convective flow ($\boldsymbol{u} = 0$) and the flux is specified with respect to a domain fixed in time (so $\boldsymbol{u}_b = 0$). In this case the concentration c_k is governed by

$$\frac{\partial c_k}{\partial t} + \nabla\cdot\mathbf{J}_k = 0, \tag{27}$$

where the constitutive law for the flux, in this case the mass flux $\mathbf{J}_k$, is typically taken to be proportional to the negative chemical potential gradient $-\nabla\mu_k$ associated with species k. Alternatively, one may similarly express the transport equation in terms of a different composition variable. A suitable characterization of the chemical potential in terms of composition (and possibly other field variables such as temperature) then completes the transport equation. To describe the transport of a given chemical system characterized by a specific Gibbs free energy (for example, those by Elliott et al. [30,32] or Landau and Lifshitz [66,67] studied in cryobiology) it is necessary to have expressions for chemical potential gradients in terms of the Gibbs free energy and the composition variables with which it is expressed.

The following sections begin with a classical thermodynamic discussion of the Gibbs free energy and its molar form. Keeping in mind the constitutive law for the transport equation, we next derive the corresponding forms for the chemical potential gradients in terms of a general Gibbs free energy function. Finally, we conclude this part of the series with a specific choice of the constitutive model of Gibbs free energy relevant to applications in cryobiology. Parallel forms of Gibbs free energy and chemical potential gradients are included in Online supplemental information.

4. Gibbs free energy

The Gibbs energy or Gibbs free energy is a form of the system energy obtained via Legendre transforms to replace entropy S and volume V with temperature T and pressure P (see for example the textbook by Callen [14]). We will use this energy as a "jumping off point" to relate the temperature, pressure, and composition of the system. If we denote the Gibbs free energy by $G = G(T, P, N_1, N_2, \ldots, N_n)$, we then can derive the differential relationship

$$\begin{aligned} dG &= \left(\tfrac{\partial G}{\partial T}\right)_{P,N_k} dT + \left(\tfrac{\partial G}{\partial P}\right)_{T,N_k} dP + \sum_{k=1}^{n}\left(\tfrac{\partial G}{\partial N_k}\right)_{T,P,N_{j\neq k}} dN_k, \\ &= -SdT + VdP + \sum_{k=1}^{n}\mu_k dN_k, \end{aligned} \tag{28}$$

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where

$$S = -\left(\frac{\partial G}{\partial T}\right)_{P,N_k}, \quad V = \left(\frac{\partial G}{\partial P}\right)_{T,N_k}, \text{ and } \mu_k = \left(\frac{\partial G}{\partial N_k}\right)_{T,P,N_{j\neq k}}. \tag{29}$$

Note that S, V and chemical potential of species k, μ_k, by definition also may depend on $T, P, N_1, \ldots, N_n$. Since G is an extensive quantity we have the Euler equation

$$G = \sum_{k=1}^{n} N_k \left(\frac{\partial G}{\partial N_k}\right)_{T,P,N_{j\neq k}} = \sum_{k=1}^{n} N_k \mu_k. \tag{30}$$

Compare this result with Eq. (7). The differential of Eq. (30) is

$$dG = \sum_{k=1}^{n} (N_k d\mu_k + \mu_k dN_k). \tag{31}$$

Subtracting Eq. (28) leads to the Gibbs–Duhem equation

$$0 = -SdT + VdP - \sum_{k=1}^{n} N_k d\mu_k. \tag{32}$$

Since S is an extensive property analogous to Eq. (7) we also have that

$$S = \sum_{k=1}^{n} \bar{S}_k N_k, \tag{33}$$

where the partial molar entropy of component k is

$$\bar{S}_k = \left(\frac{\partial S}{\partial N_k}\right)_{T,P,N_{j\neq k}}. \tag{34}$$

5. Gibbs free energy: molar free energy

An alternative to the extensive quantity G is the intensive quantity G_M representing the molar free energy (e.g. Dantzig et al. [19])

$$G_M(T, P, x_2, x_3, \ldots, x_n) := \frac{G(T, P, N_1, N_2, \ldots, N_n)}{\sum_{i=1}^{n} N_i}, \tag{35}$$

where the molar free energy depends on mole fractions x_2 through x_n as the solvent x_1 is eliminated using $\sum_{k=1}^{n} x_k = 1$ following the cryobiological standard. It also follows from Eq. (30) that

$$G_M = \frac{\sum_{k=1}^{n} \mu_k N_k}{\sum_{i=1}^{n} N_i} = \sum_{k=1}^{n} \mu_k x_k. \tag{36}$$

Differentiating G_M with respect to T or P, gives the molar entropy S_M and molar volume V_M respectively:

$$\left(\frac{\partial G_M}{\partial T}\right)_{P,x_k} = -\frac{S}{\sum_{i=1}^{n} N_i} =: -S_M, \tag{37}$$

$$\left(\frac{\partial G_M}{\partial P}\right)_{T,x_k} = \frac{V}{\sum_{i=1}^{n} N_i} =: V_M. \tag{38}$$

To identify forms for chemical potentials we differentiate G_M with respect to x_k. This is most easily accomplished by differentiating G in Eq. (35) with respect to N_k and applying the chain rule. In particular, differentiating G with respect to N_1, recognizing that $x_2, \ldots, x_n$ all depend on N_1, and using (29) leads to the result

$$\mu_1(T, P, x_2, x_3, \ldots, x_n) = G_M - \sum_{i=2}^{n} x_i \left(\frac{\partial G_M}{\partial x_i}\right)_{T,P,x_{j\neq k}}. \tag{39}$$

Differentiating G with respect to N_k for $k \neq 1$ leads to a similar result with one additional term

$$\mu_k(T, P, x_2, x_3, \ldots, x_n) = \mu_1 + \left(\frac{\partial G_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}, \quad \text{for } k = 2, \ldots, n. \tag{40}$$

The expressions for μ_1 and μ_k are equivalent to Eqs. (11) in Dantzig et al. subject to the appropriate re-interpretation of the indices ($1 \to n$ and $2, 3, \ldots, n \to 1, 2, \ldots, n-1$). These results for G_M allow us to write

$$\begin{aligned} dG_M &= \left(\frac{\partial G_M}{\partial T}\right)_{P,x_k} dT + \left(\frac{\partial G_M}{\partial P}\right)_{T,x_k} dP + \sum_{k=2}^{n} \left(\frac{\partial G_M}{\partial x_k}\right)_{T,P,x_{j\neq k}} dx_k, \\ &= -S_M dT + V_M dP + \sum_{k=2}^{n} (\mu_k - \mu_1) dx_k. \end{aligned} \tag{41}$$

The corresponding Gibbs–Duhem equation [compare Eq. (32)] is

$$0 = -S_M dT + V_M dP - \sum_{k=1}^{n} x_k d\mu_k. \tag{42}$$

Note that just as the molar volume V_M is related to partial molar volumes through Eq. (11) and likewise the molar free energy G_M is related to the chemical potentials through Eq. (36) we have that

$$S_M = \frac{S}{\sum_{i=1}^{n} N_i} = \frac{\sum_{k=1}^{n} \bar{S}_k N_k}{\sum_{i=1}^{n} N_i} = \sum_{k=1}^{n} x_k \bar{S}_k. \tag{43}$$

If we now apply to V_M and S_M [in Eqs. (37) and (38)] the same differentiation steps just applied to G_M (differentiating with respect to N_k) we find by analogy that

$$\bar{V}_1(T, P, x_2, x_3, \ldots, x_n) = V_M - \sum_{i=2}^{n} x_i \left(\frac{\partial V_M}{\partial x_i}\right)_{T,P,x_{j\neq i}}, \tag{44}$$

$$\bar{V}_k(T, P, x_2, x_3, \ldots, x_n) = \bar{V}_1 + \left(\frac{\partial V_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}, \quad \text{for } k = 2, \ldots, n, \tag{45}$$

and

$$\bar{S}_1(T, P, x_2, x_3, \ldots, x_n) = S_M - \sum_{i=2}^{n} x_i \left(\frac{\partial S_M}{\partial x_i}\right)_{T,P,x_{j\neq i}}, \tag{46}$$

$$\bar{S}_k(T, P, x_2, x_3, \ldots, x_n) = \bar{S}_1 + \left(\frac{\partial S_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}, \quad \text{for } k = 2, \ldots, n. \tag{47}$$

A parallel discussion in terms of a molality form for the Gibbs free energy $G_{molal}(T, P, m_2, m_3, \ldots, m_n)$ is given in the Online supplemental material.

6. Chemical potential gradients: molar free energy

We now recall our motivation from Section 3: species transport is dependent on the diffusive flux $\mathbf{J}_k$ defined by the standard constitutive law $\mathbf{J}_k \propto -\nabla\mu_k$. With the Gibbs free energy relationships in place from Sections 4 and 5, we may now differentiate once more to arrive at the needed chemical potential gradients. As we have seen above, the composition variable will determine the form of this quantity. Here we work in terms of mole fraction x_k and give the equivalent set of results expressed in terms of molality m_k and concentration c_k in the Supplemental material.

Before we begin, it is useful to establish the quantities

$$\begin{aligned} \nabla G_M &= \left(\frac{\partial G_M}{\partial T}\right)_{P,x_j} \nabla T + \left(\frac{\partial G_M}{\partial P}\right)_{T,x_j} \nabla P + \sum_{i=2}^{n} \left(\frac{\partial G_M}{\partial x_j}\right)_{T,P,x_{j\neq i}} \nabla x_j, \\ &= -S_M \nabla T + V_M \nabla P + \sum_{i=2}^{n} \left(\frac{\partial G_M}{\partial x_j}\right)_{T,P,x_{j\neq i}} \nabla x_j, \end{aligned} \tag{48}$$

and

$$\nabla\left(\frac{\partial G_M}{\partial x_i}\right)_{T,P,x_{j\neq i}} = -\left(\frac{\partial S_M}{\partial x_i}\right)_{T,P,x_{j\neq i}} \nabla T + \left(\frac{\partial V_M}{\partial x_i}\right)_{T,P,x_{j\neq i}} \nabla P + \sum_{j=2}^{n} \left(\frac{\partial^2 G_M}{\partial x_i \partial x_j}\right)_{T,P,x_{k\neq i,k\neq j}} \nabla x_j. \tag{49}$$

We first compute the gradient of μ_1 in Eq. (39). We find that

$$\begin{aligned}\nabla\mu_1 &= \nabla G_M - \sum_{i=2}^{n}\nabla x_i\left(\frac{\partial G_M}{\partial x_i}\right)_{T,P,x_{j\neq i}} - \sum_{i=2}^{n} x_i\nabla\left(\frac{\partial G_M}{\partial x_i}\right)_{T,P,x_{j\neq i}},\\ &= \left(-S_M\nabla T + V_M\nabla P + \sum_{j=2}^{n}\left(\frac{\partial G_M}{\partial x_j}\right)_{T,P,x_{j\neq i}}\nabla x_j\right)\\ &\quad - \sum_{i=2}^{n}\nabla x_i\left(\frac{\partial G_M}{\partial x_i}\right)_{T,P,x_{j\neq i}} - \sum_{i=2}^{n} x_i\left(-\left(\frac{\partial S_M}{\partial x_i}\right)_{T,P,x_{j\neq i}}\nabla T\right.\\ &\quad \left.+\left(\frac{\partial V_M}{\partial x_i}\right)_{T,P,x_{j\neq i}}\nabla P + \sum_{j=2}^{n}\left(\frac{\partial^2 G_M}{\partial x_i\partial x_j}\right)_{T,P,x_{k\neq i,k\neq j}}\nabla x_j\right),\\ &= \left(-S_M + \sum_{i=2}^{n} x_i\left(\frac{\partial S_M}{\partial x_i}\right)_{T,P,x_{j\neq i}}\right)\nabla T + \left(V_M - \sum_{i=2}^{n} x_i\left(\frac{\partial V_M}{\partial x_i}\right)_{T,P,x_{j\neq i}}\right)\nabla P\\ &\quad - \sum_{i=2}^{n}\sum_{j=2}^{n} x_i\left(\frac{\partial^2 G_M}{\partial x_i\partial x_j}\right)_{T,P,x_{k\neq i,k\neq j}}\nabla x_j,\\ &= -\bar{S}_1\nabla T + \bar{V}_1\nabla P - \sum_{i=2}^{n}\sum_{j=2}^{n} x_i\left(\frac{\partial^2 G_M}{\partial x_i\partial x_j}\right)_{T,P,x_{k\neq i,k\neq j}}\nabla x_j. \end{aligned} \tag{50}$$

For $k = 2, 3, \ldots, n$, we differentiate Eq. (40) to get

$$\begin{aligned}\nabla\mu_k &= \nabla\mu_1 + \nabla\left(\frac{\partial G_M}{\partial x_k}\right)_{T,P,x_{j\neq k}},\\ &= -\bar{S}_1\nabla T + \bar{V}_1\nabla P - \sum_{i=2}^{n}\sum_{j=2}^{n} x_i\left(\frac{\partial^2 G_M}{\partial x_i\partial x_j}\right)_{T,P,x_{m\neq i,k\neq j}}\nabla x_j\\ &\quad - \left(\frac{\partial S_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}\nabla T + \left(\frac{\partial V_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}\nabla P + \sum_{j=2}^{n}\left(\frac{\partial^2 G_M}{\partial x_k\partial x_j}\right)_{T,P,x_{m\neq j,m\neq k}}\nabla x_j,\\ &= \left(-\bar{S}_1 - \left(\frac{\partial S_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}\right)\nabla T + \left(\bar{V}_1 + \left(\frac{\partial V_M}{\partial x_k}\right)_{T,P,x_{j\neq k}}\right)\nabla P\\ &\quad + \sum_{j=2}^{n}\left(\left(\frac{\partial^2 G_M}{\partial x_j\partial x_k}\right)_{T,P,x_{i\neq j,i\neq k}} - \sum_{i=2}^{n} x_i\left(\frac{\partial^2 G_M}{\partial x_i\partial x_j}\right)_{T,P,x_{m\neq i,m\neq k}}\right)\nabla x_j,\\ &= -\bar{S}_k\nabla T + \bar{V}_k\nabla P + \sum_{j=2}^{n}\left(\left(\frac{\partial^2 G_M}{\partial x_j\partial x_k}\right)_{T,P,x_{m\neq j,m\neq k}} - \sum_{i=2}^{n} x_i\left(\frac{\partial^2 G_M}{\partial x_i\partial x_j}\right)_{T,P,x_{m\neq i,m\neq j}}\right)\nabla x_j. \end{aligned} \tag{51}$$

Ternary Case. Cryobiological solutions of interest are frequently ternary mixtures, containing a salt (e.g. NaCl or KCl), a CPA (e.g. Me_2SO, glycerol, etc), in the solvent water. In part III of this series, we derive and analyse a specific ternary system, therefore we present chemical potentials and their gradients here for reference. In particular, using mole fractions x_1, x_2 and x_3 and $G_M = G_M(T, P, x_2, x_3)$, we have

$$\mu_1(T,P,x_2,x_3) = G_M - x_2\left(\frac{\partial G_M}{\partial x_2}\right)_{T,P,x_1,x_2} - x_3\left(\frac{\partial G_M}{\partial x_3}\right)_{T,P,x_1,x_2}, \tag{52}$$

$$\begin{aligned}\mu_2(T,P,x_2,x_3) &= G_M + (1-x_2)\left(\frac{\partial G_M}{\partial x_2}\right)_{T,P,x_1,x_3}\\ &\quad - x_3\left(\frac{\partial G_M}{\partial x_3}\right)_{T,P,x_1,x_2}, \end{aligned} \tag{53}$$

$$\mu_3(T,P,x_2,x_3) = G_M - x_2\left(\frac{\partial G_M}{\partial x_2}\right)_{T,P,x_1,x_3} + (1-x_3)\left(\frac{\partial G_M}{\partial x_3}\right)_{T,P,x_1,x_2}. \tag{54}$$

Further, using the gradient formulas given above we have

$$\nabla\mu_k = -\bar{S}_k\nabla T + \bar{V}_k\nabla P + D_{k2}\nabla x_2 + D_{k3}\nabla x_3, \quad k = 1,2,3, \tag{55}$$

where

$$D_{12} = -x_2\left(\frac{\partial^2 G_M}{\partial x_2^2}\right)_{T,P,x_1,x_3} - x_3\left(\frac{\partial^2 G_M}{\partial x_2\partial x_3}\right)_{T,P,x_1}, \tag{56}$$

$$D_{13} = -x_2\left(\frac{\partial^2 G_M}{\partial x_2\partial x_3}\right)_{T,P,x_1} - x_3\left(\frac{\partial^2 G_M}{\partial x_3^2}\right)_{T,P,x_1,x_2}, \tag{57}$$

$$D_{22} = (1-x_2)\left(\frac{\partial^2 G_M}{\partial x_2^2}\right)_{T,P,x_1,x_3} - x_3\left(\frac{\partial^2 G_M}{\partial x_2\partial x_3}\right)_{T,P,x_1}, \tag{58}$$

$$D_{23} = (1-x_2)\left(\frac{\partial^2 G_M}{\partial x_2\partial x_3}\right)_{T,P,x_1} - x_3\left(\frac{\partial^2 G_M}{\partial x_3^2}\right)_{T,P,x_1,x_2}, \tag{59}$$

$$D_{32} = (1-x_3)\left(\frac{\partial^2 G_M}{\partial x_2\partial x_3}\right)_{T,P,x_1} - x_2\left(\frac{\partial^2 G_M}{\partial x_2^2}\right)_{T,P,x_1,x_3}, \tag{60}$$

$$D_{33} = (1-x_3)\left(\frac{\partial^2 G_M}{\partial x_3^2}\right)_{T,P,x_1,x_2} - x_2\left(\frac{\partial^2 G_M}{\partial x_2\partial x_3}\right)_{T,P,x_1}. \tag{61}$$

The general forms identified here for the chemical potential gradients in terms of temperature, pressure and composition variables are needed to define the mass flux in transport equations. Once a particular form for the Gibbs free energy is chosen the coefficients in these expressions can be determined. In the next section we present one possible form for the Gibbs free energy appropriate for use in cryobiological applications.

7. Constitutive model for Gibbs free energy

Until now we have worked with the Gibbs free energy as an abstract quantity. The selection of appropriate Gibbs Energy for any specific system is a challenge. Here we present a Gibbs energy model that is well suited to the situations encountered in cryobiology. In particular, we outline the energy used by Elliott et al. [30,32]. In the Supplemental material we present a parallel discussion of a similar free energy described by Landau and Lifshitz [66,67] and highlight the differences between the two. As with most constitutive models, there are compromises that must be made in the name of simplicity and ease of calculation, and in the context of cryopreservation it remains to be demonstrated whether there is any advantage of one Gibbs energy model choice over another.

7.1. Elliott et al. Gibbs free energy

Elliott et al. [30,32] proposed a Gibbs free energy for problems of interest in cryobiology for ternary systems. In particular, Eqs. (7)–(9) in Elliott et al. [30], with index 1 representing the solvent and 2 and 3 the solutes, are expressed here as

$$\begin{aligned}G(T,P,N_1,N_2,N_3) &= N_1\mu_1^*(T,P) + N_2\psi_2(T,P) + N_3\psi_3(T,P)\\ &\quad + N_1RT\ln\left(\frac{N_1}{N_1+N_2+N_3}\right)\\ &\quad + N_2RT\ln\left(\frac{N_2}{N_1+N_2+N_3}\right)\\ &\quad + N_3RT\ln\left(\frac{N_3}{N_1+N_2+N_3}\right)\\ &\quad + \frac{\omega_{12}N_1N_2}{N_1+N_2+N_3} + \frac{\omega_{13}N_1N_3}{N_1+N_2+N_3} + \frac{\omega_{23}N_2N_3}{N_1+N_2+N_3} \end{aligned} \tag{62}$$

where μ_1^* is the chemical potential of the pure solvent, ψ_j is a function of T and P corresponding to the limit of 'infinite dilution' of solute j, R is the universal gas constant and ω_{ij} are the interchange energies of species i with species j. One could interpret ψ_j as the chemical potential of pure solute j although the expression is not intended to be used in the extremely non-dilute setting.

The above expression can be translated to give the corresponding form for the molar free energy. In particular, using Eqs. (35) and (62) we find that

$$\begin{aligned} G_M &= x_1\mu_1^*(T,P) + x_2\psi_2(T,P) + x_3\psi_3(T,P) \\ &+ RT(x_1\ln x_1 + x_2\ln x_2 + x_3\ln x_3) \\ &+ \omega_{12}x_1x_2 + \omega_{13}x_1x_3 + \omega_{23}x_2x_3. \end{aligned} \tag{63}$$

Finally, we may differentiate Eq. (63) again to see that the chemical potentials corresponding to those given in Eqs. (52)–(54) with the Gibbs energy defined by Elliott et al. [30]:

$$\begin{aligned} \mu_1 = \; & \mu_1^* + RT\ln(1-x_2-x_3) \\ & + \omega_{12}x_2(x_2+x_3) + \omega_{13}x_3(x_2+x_3) - \omega_{23}x_2x_3, \end{aligned} \tag{64}$$

$$\begin{aligned} \mu_2 = \; & \psi_2 + RT\ln x_2 \\ & + \omega_{12}(1-x_2)(1-x_2-x_3) - \omega_{13}x_3(1-x_2-x_3) \\ & + \omega_{23}x_3(1-x_2), \end{aligned} \tag{65}$$

$$\begin{aligned} \mu_3 = \; & \psi_3 + RT\ln x_3 \\ & - \omega_{12}x_2(1-x_2-x_3) + \omega_{13}(1-x_3)(1-x_2-x_3) \\ & + \omega_{23}x_2(1-x_3), \end{aligned} \tag{66}$$

where we have eliminated the variable x_1 using $x_1 + x_2 + x_3 = 1$. The expressions for $\nabla\mu_1$, $\nabla\mu_2$ and $\nabla\mu_3$ are given by Eqs. (55) where

$$D_{12} = -\frac{RT}{1-x_2-x_3} + 2\omega_{12}x_2 + (\omega_{12}+\omega_{13}-\omega_{23})x_3, \tag{67}$$

$$D_{13} = -\frac{RT}{1-x_2-x_3} + 2\omega_{13}x_3 + (\omega_{12}+\omega_{13}-\omega_{23})x_2, \tag{68}$$

$$D_{22} = \frac{RT}{x_2} - 2\omega_{12}(1-x_2) + (\omega_{12}+\omega_{13}-\omega_{23})x_3, \tag{69}$$

$$D_{23} = 2\omega_{13}x_3 - (\omega_{12}+\omega_{13}-\omega_{23})(1-x_2), \tag{70}$$

$$D_{32} = 2\omega_{12}x_2 - (\omega_{12}+\omega_{13}-\omega_{23})(1-x_3), \tag{71}$$

$$D_{33} = \frac{RT}{x_3} + (\omega_{12}+\omega_{13}-\omega_{23})x_2 - 2\omega_{13}(1-x_3), \tag{72}$$

and using Eqs. (37), (38) along with (44)–(47) we get

$$\overline{S}_1 = -\left(\frac{\partial\mu_1^*}{\partial T}\right)_{P,x_j} - R\ln(1-x_2-x_3), \quad \overline{V}_1 = \left(\frac{\partial\mu_1^*}{\partial P}\right)_{T,x_j}, \tag{73}$$

$$\overline{S}_2 = -\left(\frac{\partial\psi_2}{\partial T}\right)_{P,x_j} - R\ln x_2, \quad \overline{V}_2 = \left(\frac{\partial\psi_2}{\partial P}\right)_{T,x_j}, \tag{74}$$

$$\overline{S}_3 = -\left(\frac{\partial\psi_3}{\partial T}\right)_{P,x_j} - R\ln x_3, \quad \overline{V}_3 = \left(\frac{\partial\psi_3}{\partial P}\right)_{T,x_j}. \tag{75}$$

Here for simplicity we have assumed that the ω_{ij} are constants. The forms for $\nabla\mu_1$, $\nabla\mu_2$ and $\nabla\mu_3$ can also be obtained by directly computing the gradient of the expressions in Eqs. (64)–(66).

8. Discussion

Understanding and predicting biological and physical phenomena at low temperatures requires models that are dominated by heat and mass transport. The goal of this series of manuscripts is to lay the foundation of transport modeling in cryobiology. As our principal motivating example, we note that Mazur's model [85] demonstrated that the intracellular state during equilibrium/quasi-equilibrium cooling is determined by the movement of water out of the cell and, while not accounted for in Eq. (1) potentially the movement of solutes into the cell. This intracellular state at any given temperature determines the likelihood of damage, classically attributed to intracellular ice formation, though recent work has called some of this into question [110]. A more complicated motivating example, the velocity of the solidification front away from or inside the cell, is limited by the diffusivity of water, the latent heat of crystallization, among other quantities (see, e.g. [59] for a review, and Toner et al. [118] and Karlsson et al. [47] for intracellular applications). In fact, Körber et al. [59–61,123] among others have demonstrated that there is solute polarization at the advancing ice front that will affect the local concentration at the cell membrane, in turn affecting the cellular state, and Levin et al. demonstrate that there is an effect of intracellular solute polarization in erythrocytes at high enough cooling rates [77].

The thermodynamical quantities describing transport are governed by the energy of the system [13]. A formulation of the energy amenable to our present purposes is the Gibbs energy, differentials of which yield expressions of the potentials that drive transport. Therefore, it is appropriate to our purpose of building the foundation of modeling in cryobiology that after clearing up the notation of compositional quantities,[6] we derive the transport equation (Eq. (27)), though this equation remains unused until the next part in our series. In this manuscript we then defined the Gibbs energy in terms of the concentrations of an arbitrary number of solutes and derived its critical differentials. We have also highlighted possibilities for composition variables and their end-effects on the constitutive equations that determine the diffusive flux in the Online supplemental material. While algebraically equivalent, the practical effects of the choices for compositional variables and constitutive equations will become more apparent in the companion papers and their supplemental material. With the exception of a few other foundational manuscripts (e.g. [10,28,30,32]) most manuscripts describing cryobiological transport modeling begin with a declaration of both the governing flux model and the chemical potential model. In this manuscript we have taken one step back from this to acknowledge that the chemical potential model is dependent on the underlying choice of energy model.

One interesting outcome of this approach is that beyond the standard concentration dependence, it yields the temperature and pressure gradient dependence of the chemical potential gradients that drive solute transport. These gradients are often overlooked or assumed negligible in the single cell cryobiological setting. We will in future manuscripts examine these assumptions more critically. Additionally, this approach allows the explicit determination of the concentration dependence of the spiecies diffusivity coefficients (i.e. Eqs. (56)–(61)) for the arbitrary Gibbs energy, and Eqs. (56)–(61) for the Gibbs energy chosen by Elliott et al. [30,32].

There have been a great number of modeling approaches in cryobiology over the last 50 years that take into considerations various aspects of the process of cooling and warming cells and tissues to and from low temperatures. The majority of manuscripts focusing on the behavior of cells in the cryobiological context assume spatial homogeneity. That is, most models of single cell cryopreservation (i.e. cells in suspension as opposed to multicellular tissue models) make the assumption that the intra- and extracellular spaces are perfectly stirred, heat transport is instantaneous, and therefore all mass transport is governed by a system of ordinary differential equations defined by transmembrane concentration or chemical potential differences. While not complete in their description of the processes, the predictions of these models have been extremely useful in guiding experimental design, demonstrating feasibility of approaches, etc. [11,12,20,36,44,48–50,69,78,84,122]. Many advances in computational complexity, transport and solution theories [10,28,32,57,97], as well as experimental

[6] For a description of the conversion among compositional variables with examples, see [57].

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parameter identification techniques [24–26,58,116,117] have been made along the way, facilitating more accurate models and better predictions of intracellular ice formation. However, interesting and potentially critical phenomena are hiding in the spatial homogeneity assumption. To address this, in their multipart analyses of intracellular solute polarization, Levin et al. [74,77] look at a "one dimensional cell" with a moving semipermeable membrane, but, while they include multiple solutes, they do not address transmembrane solute transport. While the extracellular concentrations are assumed to be given, their treatment has many of the features of the models that we will attempt to develop here. On the other side of the membrane, Körber et al. [60] carefully examine the interaction of inert particles with a solidifying ice front, setting the foundation for Chang et al. [15]. In both cases, however, the cell is non-reactive, and the diffusion within the cells and these effects on the extracellular environment are ignored. In some sense, in this series we will attempt to put together both of these models while accounting for additional effects.

In the next part of this series, we discuss bulk (away from liquid/cell and liquid/ice interfaces) and interfacial mass and energy balance equations, make specific choices for constitutive flux terms, and present equations for melting point depression as a function of concentration and pressure. In the final part, we distill the complete model derived in Part 2 into a working model that can be used to demonstrate some interesting features often unaccounted for in past and present cryobiological models.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cryobiol.2014.09.004.

References

[1] A. Abazari, J.A.W. Elliott, G. Law, L. McGann, A biomechanical triphasic approach to the transport of nondilute solutions in articular cartilage, Biophys. J . 97 (12) (2009) 3054–3064.
[2] A. Abazari, R.B. Thompson, J.A.W. Elliott, L.E. McGann, Transport phenomena in articular cartilage cryopreservation as predicted by the modified triphasic model and the effect of natural inhomogeneities, Biophys. J. 102 (6) (2012) 1284–1293.
[3] J.P. Acker, J.A.W. Elliott, L.E. McGann, Intercellular ice propagation: experimental evidence for ice growth through membrane pores, Biophys. J. 81 (3) (2001) 1389–1397.
[4] J.P. Acker, J. Pasch, I. Heschel, G. Rau, L.E. McGann, Comparison of optical measurement and electrical measurement techniques for the study of osmotic responses of cell suspensions, CryoLetters 20 (1999) 315–324.
[5] J.-O. Andersson, J. Agren, Models for numerical treatment of multicomponent diffusion in simple phases, J. Appl. Phys. 72 (4) (1992) 1350–1355.
[6] C.A. Angell, H. Senapati, Crystallization and vitrification in cryoprotected aqueous systems, in: D.E. Pegg, A.M. Karow Jr. (Eds.), The Biophysics of Organ Cryopreservation, NATO ASI Series, vol. 147, Plenum Press, New York, 1987, pp. 147–171.
[7] P.H. Barry, J.M. Diamond, Effects of unstirred layers on membrane phenomena, Physiol. Rev. 64 (3) (1984) 763–872.
[8] R.P. Batycky, R. Hammerstedt, D.A. Edwards, Osmotically driven intracellular transport phenomena, Philos. Trans. R. Soc. Lond. Ser. A Math. Phys. Eng. 355 (1734) (1997) 2459–2487.
[9] C.T. Benson, C. Liu, D.Y. Gao, E.S. Critser, J. Benson, J. Critser, Hydraulic conductivity (Lp) and its activation energy (Ea), cryoprotectant agent permeability (Ps) and its Ea, and reflection coefficients (sigma) for golden hamster individual pancreatic islet cell membranes, Cryobiology 37 (4) (1998) 290–299.
[10] J.D. Benson, Stability analysis of several non-dilute multiple solute transport equations, J. Math. Chem. 49 (4) (2011) 859–869.
[11] J.D. Benson, C.C. Chicone, J.K. Critser, A general model for the dynamics of cell volume, global stability and optimal control, J. Math. Biol. 63 (2) (2011) 339–359.
[12] J.D. Benson, C.C. Chicone, J.K. Critser, Analytical optimal controls for the state constrained addition and removal of cryoprotective agents, Bull. Math. Biol. 74 (2012) 1516–1530.
[13] R.B. Bird, W.E. Stewart, E.N. Lightfoot, Transport Phenomena, second ed., John Wiley & Sons, 2002.
[14] H.B. Callen, Thermodynamics and An Introduction to Thermostatics, 2nd ed., John Wiley & Sons Inc., New York, 1985.
[15] A. Chang, J.A. Dantzig, B.T. Darr, A. Hubel, Modeling the interaction of biological cells with a solidifying interface, J. Comput. Phys. 226 (2) (2007) 1808–1829.
[16] A. Chaveiro, J. Liu, B. Engel, J.K. Critser, H. Woelders, Significant variability among bulls in the sperm membrane permeability for water and glycerol: possible implications for semen freezing protocols for individual males, Cryobiology 53 (3) (2006) 349–359.
[17] E. Cohen, T. Cvitas, J. Frey, B. Holmstram, K. Kuchitsu, Â.R. Marquardt, I. Mills, F. Pavese, M. Quack, J. Stohner, Â.H. Strauss, M. Takami, A. Thor, Quantities, Units and Symbols in Physical Chemistry, Springer Verlag, 2007.
[18] M. Conti, Planar solidification of a finite slab: effects of the pressure dependence of the freezing point, Int. J. Heat Mass Transfer 38 (1) (1995) 65–70.
[19] J.A. Dantzig, W.J. Boettinger, J.A. Warren, G.B. McFadden, S.R. Coriell, R.F. Sekerka, Numerical modeling of diffusion-induced deformation, Metall. Mater. Trans. A 37A (2006) 2701–2714.
[20] A.F. Davidson, J.D. Benson, A.Z. Higgins, Mathematically optimized cryoprotectant equilibration procedures for cryopreservation of human oocytes, Theor. Biol. Med. Modell. 11 (2014) 13.
[21] S.H. Davis, Theory of Solidification, Cambridge University Press, 2001.
[22] R.V. Devireddy, Statistical thermodynamics of biomembranes, Cryobiology 60 (2010) 80–90.
[23] R.V. Devireddy, P.R. Barratt, K.B. Storey, J.C. Bischof, Liver freezing response of the freeze-tolerant wood frog, rana sylvatica, in the presence and absence of glucose. ii. Mathematical modeling, Cryobiology 38 (4) (1999) 327–338.
[24] R.V. Devireddy, B. Fahrig, R.A. Godke, S.P. Leibo, Subzero water transport characteristics of boar spermatozoa confirm observed optimal cooling rates, Mol. Reprod. Dev. 67 (4) (2004) 446–457.
[25] R.V. Devireddy, D. Raha, J.C. Bischof, Measurement of water transport during freezing in cell suspensions using a differential scanning calorimeter, Cryobiology 36 (2) (1998) 124–155.
[26] R.V. Devireddy, D.J. Swanlund, A.S. Alghamdi, L.A. Duoos, M.H.T. Troedsson, J.C. Bischof, K.P. Roberts, Measured effect of collection and cooling conditions on the motility and the water transport parameters at subzero temperatures of equine spermatozoa, Reproduction 124 (5) (2002) 643–648.
[27] D.P. Eisenberg, M.J. Taylor, Y. Rabin, Thermal expansion of the cryoprotectant cocktail DP6 combined with synthetic ice modulators in presence and absence of biological tissues, Cryobiology 65 (2012) 117–125.
[28] J.A.W. Elliott, H.Y. Elmoazzen, L.E. McGann, A method whereby Onsager coefficients may be evaluated, J. Chem. Phys. 113 (16) (2000) 6573–6578.
[29] J.A.W. Elliott, S.S.L. Peppin, Particle trapping and banding in rapid colloidal solidification, Phys. Rev. Lett. 107 (16) (2011) 168301.
[30] J.A.W. Elliott, R.C. Prickett, H.Y. Elmoazzen, K.R. Porter, L.E. McGann, A multisolute osmotic virial equation for solutions of interest in biology, J. Phys. Chem. B 111 (7) (2007) 1775–1785.
[31] H.Y. Elmoazzen, J.A.W. Elliott, L.E. McGann, Cryoprotectant equilibration in tissues, Cryobiology 51 (1) (2005) 85–91.
[32] H.Y. Elmoazzen, J.A.W. Elliott, L.E. McGann, Osmotic transport across cell membranes in nondilute solutions: a new nondilute solute transport equation, Biophys. J. 96 (7) (2009) 2559–2571.
[33] D.L. Feltham, J. Garside, Analytical and numerical solutions describing the inward solidification of a binary melt, Chem. Eng. Sci. 56 (2001) 2357–2370.
[34] D.Y. Gao, C.T. Benson, C. Liu, J.J. McGrath, E.S. Critser, J.K. Critser, Development of a novel microperfusion chamber for determination of cell membrane transport properties, Biophys. J . 71 (1) (1996) 443–450.
[35] D.Y. Gao, Q. Chang, C. Liu, K. Farris, K. Harvey, L.E. McGann, D. English, J. Jansen, J.K. Critser, Fundamental cryobiology of human hematopoietic progenitor cells i: Osmotic characteristics and volume distribution, Cryobiology 36 (1) (1998) 40–48.
[36] J.A. Gilmore, J. Liu, D.Y. Gao, J.K. Critser, Determination of optimal cryoprotectants and procedures for their addition and removal from human spermatozoa, Hum. Reprod. 12 (1997) 112–118.
[37] J.A. Gilmore, J. Liu, A.T. Peter, J.K. Critser, Determination of plasma membrane characteristics of boar spermatozoa and their relevance to cryopreservation, Biol. Reprod. 58 (1) (1998) 28–36.
[38] J.A. Gilmore, L.E. McGann, J. Liu, D.Y. Gao, A.T. Peter, F.W. Kleinhans, J.K. Critser, Effect of cryoprotectant solutes on water permeability of human spermatozoa, Biol. Reprod. 53 (5) (1995) 985–995.
[39] A.I. Glazar, S.F. Mullen, J. Liu, J.D. Benson, J.K. Critser, E.L. Squires, J.K. Graham, Osmotic tolerance limits and membrane permeability characteristics of stallion spermatozoa treated with cholesterol, Cryobiology 59 (2) (2009) 201–206.
[40] H.R. Harmison, K.R. Diller, J.R. Walsh, C.M. Neils, J.J. Brand, Measurement of cell volume loss in the liquid region preceding an advancing phase change interfacea, Ann. N. Y. Acad. Sci. 858 (1) (1998) 276–283.
[41] A. Hubel, T.B. Darr, A. Chang, J. Dantzig, Cell partitioning during the directional solidification of trehalose solutions, Cryobiology 55 (3) (2007) 182–188.

[42] M. Jacobs, The simultaneous measurement of cell permeability to water and to dissolved substances, J. Cell. Comp. Physiol. 2 (1932) 427–444.
[43] M. Jaeger, M. Carin, M. Medale, G. Tryggvason, The osmotic migration of cells in a solute gradient, Biophys. J. 77 (3) (1999) 1257–1267.
[44] J.O. Karlsson, E.A. Szurek, A.Z. Higgins, S.R. Lee, A. Eroglu, Optimization of cryoprotectant loading into murine and human oocytes, Cryobiology 68 (1) (2014) 18–28. URL: <http://www.sciencedirect.com/cryo/article/pii/S0011224013004203>.
[45] J.O.M. Karlsson, E.G. Cravalho, I.H.M.B. Rinkes, R.G. Tompkins, M.L. Yarmush, M. Toner, Nucleation and growth of ice crystals inside cultured-hepatocytes during freezing in the presence of dimethyl-sulfoxide, Biophys. J. 65 (6) (1993) 2524–2536.
[46] J.O.M. Karlsson, E.G. Cravalho, M. Toner, Intracellular ice formation: causes and consequences, Cryo Letters 14 (1993) 323–336.
[47] J.O.M. Karlsson, E.G. Cravalho, M. Toner, A model of diffusion-limited ice growth inside biological cells during freezing, J. Appl. Phys. 75 (9) (1994) 4442–4455.
[48] J.O.M. Karlsson, A. Eroglu, T.L. Toth, E.G. Cravalho, M. Toner, Fertilization and development of mouse oocytes cryopreserved using a theoretically optimized protocol, Hum. Reprod. 11 (6) (1996) 1296–1305.
[49] C.M. Kashuba, J.D. Benson, J.K. Critser, Rationally optimized cryopreservation of multiple mouse embryonic stem cell lines: I—comparative fundamental cryobiology of multiple mouse embryonic stem cell lines and the implications for embryonic stem cell cryopreservation protocols, Cryobiology 68 (2) (2014) 166–175. URL: <http://www.sciencedirect.com/cryo/article/pii/S0011224013004331>.
[50] C.M. Kashuba, J.D. Benson, J.K. Critser, Rationally optimized cryopreservation of multiple mouse embryonic stem cell lines: II—Mathematical prediction and experimental validation of optimal cryopreservation protocols, Cryobiology 68 (2) (2014) 176–184. URL: <http://www.sciencedirect.com/cryo/article/pii/S001122401300429X>.
[51] C.M. Kashuba Benson, J.D. Benson, J.K. Critser, An improved cryopreservation method for a mouse embryonic stem cell line, Cryobiology 56 (2008) 120–130.
[52] I. Katkov, A two-parameter model of cell membrane permeability for multisolute systems, Cryobiology 40 (1) (2000) 64–83.
[53] I. Kececioglu, B. Rubinsky, A continuum model for the propagation of discrete phase-change fronts in porous media in the presence of coupled heat flow, fluid flow and species transport processes, Int. J. Heat Mass Transfer 32 (6) (1989) 1111–1130.
[54] O. Kedem, A. Katchalsky, Thermodynamic analysis of the permeability of biological membranes to non-electrolytes, Biochim. Biophys. Acta 27 (2) (1958) 229–246.
[55] O. Kedem, A. Katchalsky, A physical interpretation of the phenomenological coefficients of membrane permeability, J. Gen. Physiol. 45 (1961) 143–179.
[56] F.W. Kleinhans, Membrane permeability modeling: Kedem–Katchalsky vs a two-parameter formalism, Cryobiology 37 (4) (1998) 271–289.
[57] F.W. Kleinhans, P. Mazur, Comparison of actual vs. synthesized ternary phase diagrams for solutes of cryobiological interest, Cryobiology 54 (2) (2007) 212–222.
[58] F.W. Kleinhans, P. Mazur, Determination of the water permeability (lp) of mouse oocytes at −25 °C and its activation energy at subzero temperatures, Cryobiology 58 (2) (2009) 215–224.
[59] C. Körber, Phenomena at the advancing ice–liquid interface: solutes, particles and biological cells, Q. Rev. Biophys. 21 (02) (1988) 229–298.
[60] C. Körber, G. Rau, M.D. Cosman, E.G. Cravalho, Interaction of particles and a moving ice–liquid interface, J. Cryst. Growth 72 (3) (1985) 649–662.
[61] C. Körber, M.W. Scheiwe, K. Wollhöver, Solute polarization during planar freezing of aqueous salt solutions, Int. J. Heat Mass Transfer 26 (8) (1983) 1241–1253.
[62] R.K. Korhonen, S.-K. Han, W. Herzog, Osmotic loading of articular cartilage modulates cell deformations along primary collagen fibril directions, J. Biomech. 43 (2010) 783–787.
[63] P.L. Lacelle, A. Rothstein, The passive permeability of the red blood cell to cations, J. Gen. Physiol. 50 (1966) 171–188.
[64] K.J. Laidler, K.E. Shuler, The kinetics of membrane processes. I. The mechanism and the kinetic laws for diffusion through membranes, J. Chem. Phys. 17 (10) (1949) 851–855.
[65] K.J. Laidler, K.E. Shuler, The kinetics of membrane processes. II. Theoretical pressure–time relationships for permeable membranes, J. Chem. Phys. 17 (10) (1949) 856–860.
[66] L.D. Landau, E.M. Lifshitz, Course of Theoretical Physics. 5: Statistical Physics, second ed., Pergamon Press, 1958.
[67] L.D. Landau, E.M. Lifshitz, Statistical Physics, Course of Theoretical Physics, third ed., vol. 5, Pergamon Press, Oxford, 1980.
[68] L.G. Leal, Advanced Transport Phenomena: Fluid Mechanics of Convective Transport Processes, Cambridge University Press, 2007.
[69] R.L. Levin, A generalized method for the minimization of cellular osmotic stresses and strains during the introduction and removal of permeable cryoprotectants, J. Biomech. Eng. 104 (2) (1982) 81–86.
[70] R.L. Levin, E.G. Cravalho, C.E. Huggins, Effect of concentration polarization of solutes on survival of erythrocytes during freezing, Cryobiology 13 (6) (1976) 649–650.
[71] R.L. Levin, E.G. Cravalho, C.E. Huggins, Effect of hydration on the water content of human erythrocytes, Biophys. J. 16 (12) (1976) 1411–1426.
[72] R.L. Levin, E.G. Cravalho, C.E. Huggins, The concentration polarization effect in frozen erythrocytes, J. Biomech. Eng. Trans. ASME 99 (2) (1977) 65–73.
[73] R.L. Levin, E.G. Cravalho, C.E. Huggins, Concentration polarization of solutes within biological cells, Mech. Eng. 99 (7) (1977) 101.
[74] R.L. Levin, E.G. Cravalho, C.E. Huggins, Diffusion transport in a liquid solution with a moving, semipermeable boundary, J. Heat Transfer Trans. ASME 99 (2) (1977) 322–329.
[75] R.L. Levin, E.G. Cravalho, C.E. Huggins, Effect of solution non-ideality on erythrocyte volume regulation, Biochim. Biophys. Acta 465 (2) (1977) 179–190.
[76] R.L. Levin, E.G. Cravalho, C.E. Huggins, Water transport in a cluster of closely packed erythrocytes at subzero temperatures, Cryobiology 14 (5) (1977) 549–558.
[77] R.L. Levin, E.G. Cravalho, C.E. Huggins, The concentration polarization effect in a multicomponent electrolyte solution – human erythrocyte, J. Theor. Biol. 71 (2) (1978) 225–254.
[78] R.L. Levin, T. Miller, An optimum method for the introduction or removal of permeable cryoprotectants: isolated cells, Cryobiology 18 (1) (1981) 32–48.
[79] C. Liu, C.T. Benson, D.Y. Gao, B.W. Haag, L.E. Mcgann, J.K. Critser, Water permeability and its activation-energy for individual hamster pancreatic islet cells, Cryobiology 32 (5) (1995) 493–502.
[80] C. Liu, L.E. McGann, D.Y. Gao, B.W. Haag, J.K. Critser, Osmotic separation of pancreatic exocrine cells from crude islet cell preparations, Cell Transplant. 5 (1) (1996) 31–39.
[81] J. Liu, E.J. Woods, Y. Agca, E.S. Critser, J.K. Critser, Cryobiology of rat embryos II: A theoretical model for the development of interrupted slow freezing procedures, Biol. Reprod. 63 (5) (2000) 1303–1312.
[82] J. Liu, M.A.J. Zieger, J.R.T. Lakey, E.J. Woods, J.K. Critser, The determination of membrane permeability coefficients of canine pancreatic islet cells and their application to islet cryopreservation, Cryobiology 35 (1) (1997) 1–13.
[83] B. Lucké, M. McCutcheon, The living cell as an osmotic system and its permeability to water, Physiol. Rev. 12 (1) (1932) 68–139.
[84] R.E. Lusianti, J.D. Benson, J.P. Acker, A.Z. Higgins, Rapid removal of glycerol from frozen-thawed red blood cells, Biotechnol. Prog. 69 (2013) 609–620.
[85] P. Mazur, Kinetics of water loss from cells at subzero temperatures and the likelihood of intracellular freezing, J. Gen. Physiol. 47 (1963) 347–369.
[86] P. Mazur, Equilibrium, quasi-equilibrium, and nonequilibrium freezing of mammalian embryos, Cell Biophys. 17 (1) (1990) 53–92.
[87] P. Mazur, S. Leibo, E. Chu, A two-factor hypothesis of freezing injury. Evidence from Chinese hamster tissue-culture cells, Exp. Cell Res. 71 (2) (1972) 345–355.
[88] P. Mazur, I.L. Pinn, F.W. Kleinhans, Intracellular ice formation in mouse oocytes subjected to interrupted rapid cooling, Cryobiology 55 (2) (2007) 158–166.
[89] Y. Mori, Mathematical properties of pump-leak models of cell volume control and electrolyte balance, J. Math. Biol. 65 (5) (2012) 875–918.
[90] Y. Mori, C. Liu, R.S. Eisenberg, A model of electrodiffusion and osmotic water flow and its energetic structure, Physica D 240 (2011) 1835–1852.
[91] G.J. Morris, Rapidly cooled human sperm: no evidence of intracellular ice formation, Hum. Reprod. 21 (8) (2006) 2075–2083.
[92] G.J. Morris, E. Acton, B.J. Murray, F. Fonseca, Freezing injury: the special case of the sperm cell, Cryobiology 64 (2012) 71–80.
[93] D.E. Pegg, B. Rubinsky, M.P. Diaper, C.Y. Lee, Analysis of the introduction and removal of glycerol in rabbit kidneys using a krogh cylinder model, Cryobiology 23 (2) (1986) 150–160.
[94] S.S.L. Peppin, J.A.W. Elliott, M.G. Worster, Solidification of colloidal suspensions, J. Fluid Mech. 554 (2006) 147–166.
[95] J.A. Preciado, B. Rubinsky, Isochoric preservation: a novel characterization method, Cryobiology 60 (2010) 23–29.
[96] R.C. Prickett, J.A.W. Elliott, S. Hakda, L.E. McGann, A non-ideal replacement for the Boyle van't Hoff equation, Cryobiology 57 (2) (2008) 130–136.
[97] R.C. Prickett, J.A.W. Elliott, L.E. McGann, Application of the osmotic virial equation in cryobiology, Cryobiology 60 (1) (2010) 30–42.
[98] R.C. Prickett, J.A.W. Elliott, L.E. McGann, Application of the multisolute osmotic virial equation to solutions containing electrolytes, J. Phys. Chem. B 115 (2011) 14531–14543.
[99] Y. Rabin, J. Plitz, Thermal expansion of blood vessels and muscle specimens permeated with dmso, dp6, and vs55 at cryogenic temperatures, Ann. Biomed. Eng. 33 (9) (2005) 1213–1228.
[100] D.H. Rasmussen, A.P. MacKenzie, Effect of solute on ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperatures, in: Water structure at the water–polymer interface, Springer, 1972, pp. 126–145.
[101] N. Read, D. Barber, R. Levin, C. Holdsworth, Unstirred layer and kinetics of electrogenic glucose absorption in the human jejunum in situ, Gut. 18 (1977) 865–876.
[102] L.U. Ross-Rodriguez, J.A.W. Elliott, L.E. McGann, Investigating cryoinjury using simulations and experiments. 1: TF-1 cells during two-step freezing (rapid cooling interrupted with a hold time), Cryobiology 61 (1) (2010) 38–45. URL: <http://www.sciencedirect.com/cryo/article/pii/S0011224010000775>.
[103] L.U. Ross-Rodriguez, J.A.W. Elliott, L.E. McGann, Investigating cryoinjury using simulations and experiments: 2. TF-1 cells during graded freezing (interrupted slow cooling without hold time), Cryobiology 61 (1) (2010) 46–51. URL: <http://www.sciencedirect.com/cryo/article/pii/S0011224010000787>.

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[104] L.U. Ross-Rodriguez, J.A.W. Elliott, L.E. McGann, Non-ideal solution thermodynamics of cytoplasm, Biopreserv. Biobank. 10 (2012) 463–471.
[105] B. Rubinsky, P.A. Perez, M.E. Carlson, The thermodynamic principles of isochoric cryopreservation, Cryobiology 50 (2) (2005) 121–138.
[106] B. Rubinsky, S. Szobota, Isochoric method and device for reducing the probability of ice nucleation during preservation of biological matter at subzero centigrade temperatures, US Patent App. 11/485,922 (Feb. 22 2007). URL: <https://www.google.com/patents/US20070042337>.
[107] J. Saenz, M. Toner, R. Risco, Comparison between ideal and nonideal solution models for single-cell cryopreservation protocols, J. Phys. Chem. B 113 (2009) 4853–4864.
[108] L. Salinas-Flores, S.L. Adams, M.H. Lim, Determination of the membrane permeability characteristics of pacific oyster, crassostrea gigas, oocytes and development of optimized methods to add and remove ethylene glycol, Cryobiology 56 (1) (2008) 43–52.
[109] R.F. Sekerka, Similarity solutions for a binary diffusion couple with diffusivity and density dependent on composition, Prog. Mater Sci. 49 (2004) 511–536.
[110] S. Seki, B. Jin, P. Mazur, Extreme rapid warming yields high functional survivals of vitrified 8-cell mouse embryos even when suspended in a half-strength vitrification solution and cooled at moderate rates to 196 °C, Cryobiology 68 (1) (2014) 71–78.
[111] Z. Shaozhi, D.E. Pegg, Analysis of the permeation of cryoprotectants in cartilage, Cryobiology 54 (2) (2007) 146–153.
[112] O.M. Silvares, E.G. Cravalho, W.M. Toscano, C.E. Huggins, Thermodynamics of water transport from biological cells during freezing, J. Heat Transfer Trans. ASME 97 (4) (1975) 582–588.
[113] S.L. Sobolev, Local non-equilibrium diffusion model for solute trapping during rapid solidification, Acta Mater. 60 (6) (2012) 2711–2718.
[114] P.S. Steif, M.C. Palastro, Y. Rabin, The effect of temperature gradients on stress development during cryopreservation via vitrification, Cell Preserv. Technol. 5 (2) (2007) 104–115.
[115] G.J. Suppes, S. Egan, A.J. Casillan, K.W. Chan, B. Seckar, Impact of high pressure freezing on DH5 *Escherichia coli* and red blood cells, Cryobiology 47 (2003) 93–101.
[116] S. Thirumala, R.V. Devireddy, A simplified procedure to determine the optimal rate of freezing biological systems, J. Biomech. Eng. 127 (2005) 295–300.
[117] S. Thirumala, C. Huang, Q. Dong, T.R. Tiersch, R.V. Devireddy, A theoretically estimated optimal cooling rate for the cryopreservation of sperm cells from a live-bearing fish, the green swordtail xiphophorus helleri, Theriogenology 63 (9) (2005) 2395–2415.
[118] M. Toner, E.G. Cravalho, M. Karel, Thermodynamics and kinetics of intracellular ice formation during freezing of biological cells, J. Appl. Phys. 67 (3) (1990) 1582–1593.
[119] M. Toner, E.G. Cravalho, M. Karel, Cellular response of mouse oocytes to freezing stress: prediction of intracellular ice formation, J. Biomech. Eng. 115 (2) (1993) 169–174.
[120] V.V. Turov, S.V. Kerus, V.M. Gun'ko, Behaviour of water bound in bone marrow cells affected by organic solvents of different polarity, Cryobiology 59 (2009) 102–112.
[121] L. Weng, W. Li, C. Chen, J. Zuo, Kinetics of coupling water and cryoprotectant transport across cell membranes and applications to cryopreservation, J. Phys. Chem. B 115 (2011) 14721–14731.
[122] H. Woelders, A. Chaveiro, Theoretical prediction of 'optimal' freezing programmes, Cryobiology 49 (3) (2004) 258–271.
[123] K. Wollhöver, M. Scheiwe, U. Hartmann, C. Körber, On morphological stability of planar phase boundaries during unidirectional transient solidification of binary aqueous solutions, Int. J. Heat Mass Transfer 28 (5) (1985) 897–902.
[124] E.J. Woods, J. Liu, J.A. Gilmore, T.J. Reid, D.Y. Gao, J.K. Critser, Determination of human platelet membrane permeability coefficients using the kedem-katchalsky formalism: Estimates from two- vs three-parameter fits, Cryobiology 38 (3) (1999) 200–208, in: Symposium on Cryopreservation of Human Platelets, at the 35th Annual Meeting of the Society-for-Cryobiology, Pittsburgh, Pennsylvania, July 16, 1998.
[125] G. Yang, A. Zhang, L.X. Xu, X. He, Modeling the cell-type dependence of diffusion-limited intracellular ice nucleation and growth during both vitrification and slow freezing, J. Appl. Phys. 105 (11) (2009) 114701.
[126] X. Yu, G. Chen, S. Zhang, A model for predicting the permeation of dimethyl sulfoxide into articular cartilage, and its application to the liquidus-tracking method, Cryobiology 67 (3) (2013) 332–338.
[127] A. Zhang, L.X. Xu, G.A. Sandison, J. Zhang, A microscale model for prediction of breast cancer cell damage during cryosurgery, Cryobiology 47 (2) (2003) 143–154.
[128] A.I. Zhmakin, Physical aspects of cryobiology, Phys. Usp. 51 (3) (2008). 321-252.
[129] M.A.J. Zieger, E.E. Tredget, L.E. McGann, Mechanisms of cryoinjury and cryoprotection in split-thickness skin, Cryobiology 33 (3) (1996) 376–389.
[130] C.E. Zobell, A.B. Cobet, Growth, reproduction, and death rates of escherichia coli at increased hydrostatic pressures, J. Bacteriol. 84 (1962) 1228–1236.

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