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Foundations of modeling in cryobiology—III: Inward solidification of a ternary solution towards a permeable spherical cell in the dilute limit

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Abstract

In the previous two manuscripts we outlined the general theory of heat and mass transport in a cell-liquid-ice system with general boundaries and nonideal and nondilute assumptions. Here we simplify the models considerably by presenting a reduction to a spherically symmetric system—a spherical cell with an encroaching spherical ice front. We also reduce to linear approximations of the nonideal nondilute models, essentially assuming dilute and ideal conditions. We derive the resulting nondimensional combined heat and mass transport model for a ternary solution and present numerical solutions. We include an analysis of the effects of varying some nondimensional parameters on rates of ice growth with comments on the necessity of models that account for spatially varying quantities in cryobiology.

Keywords

Gibbs Free Energy; partition coefficient; density; diffusion; chemical potential gradient

1. Introduction

In the first two parts of this series [4, 7], a general model of the interaction between a cell, its surrounding media, and encompassing solidifying ice front was developed. The proposed model accounts for the interdependence of spatial and temporal gradients of temperature, species concentrations and pressures as well as their effects on interfacial velocities and fluxes. The modeling of these processes is complex, especially in the fully generic setting where no spatial domain assumptions and no dilute and ideal solution approximations are

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made. In fact, to our knowledge there have been no studies that account for all of the varied effects included in the model from the first two parts of this series. This may be in part due to parameter identification challenges: there are a number of relatively unstudied parameters in the models presented, especially with regards to large-scale temperature dependence. This may also be in part due to the computational complexity – double free boundary problems are inherently computationally challenging – not to mention the complications of 16 coupled physical, chemical, and biological processes.

As a means for exploration of a number of effects and to present an example of how this model can be used, here we attempt to reduce the full model to one with the minimal set of components that yield a complete model. In particular, we simplify the geometry by making a spherical symmetry assumption and we make ideal dilute solution and model assumptions that affect bulk and membrane transport as well as the freezing point depression. This allows the statement of a problem in the context of nested spheres, a one-dimensional spatial differential equation (PDE) system that may be solved via finite difference techniques. To present a meaningful yet tractable analysis of practical cryobiological relevance, we present the reduced model and its nondimensionalization, and then analyze the effects of including key model parts in terms of the cryobiologically relevant quantity of the ratio of liquid and solute densities.

We limit the external temperature function to a simple step function, where the temperature goes from an equilibrium at the liquidus temperature of the system, to a temperature below the liquidus curve. This case still has considerable cryobiological relevance as there are a number of published protocols where cells are rapidly cooled to an intermediate holding temperature, ice is nucleated, and cells are held for a fixed amount of time [27, 30].

Finally, we emphasize that in this manuscript our aim is to present a version of the complete model, not to explore and/or test all of the relative and potential effects highlighted in the first parts. We hope the presented model and its relatively brief analysis will inspire further discussion about the important quantities and parameters that may affect the accurate prediction of the cellular state during cryopreservation.

2. Model Description

We assume that the initial state of the system has liquid surrounding the cell with no solid material and that solidification begins after time $t = 0$. In all regions we assume an initial state of thermal and chemical equilibrium. This assumption, of course, can be modified in the presence of better data showing concentration or thermal gradients held by cell-media systems. In particular, our example system will consist of liquid containing the permeable cryoprotective agent (CPA) Me_2SO , and the impermeable solutes NaCl and KCl , represented by the concentrations A (extra- and intracellular), B (extracellular), and C (intracellular), respectively. We will set the initial extracellular concentrations to be A_L^{init} and B_L^{init} , and the initial system temperature T^{init} to be the liquidus temperature associated with concentrations A_L^{init} and B_L^{init} (denoted below by $T_{\mathcal{L}}^0$). In the cell the initial concentrations are $A_C^{\text{init}} = A_L^{\text{init}}$ and C_C^{init} where C_C^{init} is chosen so that the liquidus temperature in the cell is T^{init} and the

transmembrane nonpermeating solute chemical potential differences are zero (i.e. the cell is at chemical equilibrium).

We assume spherical symmetry (see Figure 1), where the cell, the surrounding liquid, and the surrounding solid regions are nested spheres, and the initial radius of the cell is given by R_C^{init} . Once solidification begins a third, solid phase is present. The cell occupies the region Ω_C where $0 < r < R_C$. The liquid occupies the region Ω_L where $R_C < r < R_I$. The solid occupies the region Ω_S where $R_I < r < R_O$. R_O is assumed to be a fixed outer container radius while R_I and R_C are the unknown positions of the solid–liquid interface and the cell membrane, respectively. In the case under consideration here, R_C and R_I are functions of time only; spherical symmetry is assumed. The variables of interest in the extracellular liquid region are the water concentration W_L , the permeating solute concentration A_L (e.g. a CPA concentration of dimethyl sulphoxide, glycerol, propylene glycol, or ethylene glycol) and the nonpermeating solute concentration B_L (e.g. a salt concentration of sodium chloride or potassium chloride). In the solidified region we have the corresponding variables W_S , A_S and B_S . The intracellular solution is made up of water, denoted by concentration W_C , the permeating solute, denoted by concentration A_C and a nonpermeating solute, denoted by concentration C_C , that in general is different from the nonpermeating solute in the extracellular solution. Since $A + B + W = 1/V_M$ (and similarly for the variables inside the cell; c.f. eq. (13) in part 1 [4]) we present the equations in terms of the solute variables A_L , B_L , A_C and C_C . In addition to these concentration variables we are also interested in the temperature T throughout the system (denoted with subscripts ‘S’, ‘L’ and ‘C’ in the solid, liquid and cell regions, respectively). In this model we assume that there is no convective fluid motion anywhere in the system, a requirement of the symmetry and incompressible fluid assumptions.

For initial values of the extracellular concentrations A_L^{init} and B_L^{init} we shall use values considered by Kleinhans and Mazur[26]. In particular, we shall base the initial non-permeating solute (salt) concentration on an isotonic NaCl solution which, when dissociated into its constituents with dissociation constant k_{diss} , gives $k_{\text{diss}} B_L^{\text{init}} = 0.3 \text{ mol/L}$. For an initial concentration of CPA we shall take $A_L^{\text{init}} = 1 \text{ mol/L}$ (see Table 3 in Kleinhans and Mazur for a range of cryobiologically-relevant CPA values). While the initial intracellular nonpermeating concentration, C_C^{init} is related via the Boyle van’t Hoff relationship [9] to R_C^{init} , it is simpler and equivalent to assume thermodynamic equilibrium at the initial temperature (see Sec. 2.2.4 below).

2.1. Bulk Transport

In the interior of Ω_S , Ω_L and Ω_C , standard diffusion equations govern the temperature and concentration. In particular, in the solid phase we have

$$\begin{aligned}
\frac{\partial T_S}{\partial t} &= \kappa^{\text{sol}} \nabla^2 T_S = \frac{\kappa^{\text{sol}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial T_S}{\partial r} \right), \\
\frac{\partial A_S}{\partial t} &= 0, \\
\frac{\partial B_S}{\partial t} &= 0,
\end{aligned} \quad R_I < r < R_O, \quad (1)$$

where κ^{sol} is the thermal diffusion coefficient in the solid phase assumed to be constant and we assume that no species diffusion occurs in the solid. In the liquid phase we have

$$\begin{aligned}
\frac{\partial T_L}{\partial t} &= \kappa^{\text{liq}} \nabla^2 T_L = \frac{\kappa^{\text{liq}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial T_L}{\partial r} \right), \\
\frac{\partial A_L}{\partial t} &= D_A^{\text{liq}} \nabla^2 A_L = \frac{D_A^{\text{liq}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial A_L}{\partial r} \right), \\
\frac{\partial B_L}{\partial t} &= D_B^{\text{liq}} \nabla^2 B_L = \frac{D_B^{\text{liq}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial B_L}{\partial r} \right),
\end{aligned} \quad R_C < r < R_I, \quad (2)$$

where κ^{liq} is the liquid thermal diffusion coefficient, and D_A^{liq} and D_B^{liq} are the solutal diffusion coefficients in the liquid for species A and B, respectively. Finally in the cell interior we have

$$\begin{aligned}
\frac{\partial T_C}{\partial t} &= \kappa^{\text{cell}} \nabla^2 T_C = \frac{\kappa^{\text{cell}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial T_C}{\partial r} \right), \\
\frac{\partial A_C}{\partial t} &= D_A^{\text{cell}} \nabla^2 A_C = \frac{D_A^{\text{cell}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial A_C}{\partial r} \right), \\
\frac{\partial C_C}{\partial t} &= D_C^{\text{cell}} \nabla^2 C_C = \frac{D_C^{\text{cell}}}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_C}{\partial r} \right)
\end{aligned} \quad 0 < r < R_C, \quad (3)$$

where κ^{cell} is the thermal diffusion coefficient in the cell, and D_A^{cell} and D_C^{cell} are the solutal diffusion coefficients in the cell for species A and C, respectively. While we treat interfacial boundary conditions below, note that boundary conditions at the center of the cell in this spherical case address boundedness for variables T_C , A_C and C_C by stating that the radial derivative is zero at $r = 0$ for these quantities.

2.2. Interfacial/Boundary and Initial Conditions

Systems (1)–(3) require associated interfacial boundary conditions. These are discussed at length in general in Part 2 of the series [7], and entail mass, momentum, energy and other balances at the cell membrane, the liquid/solid interface, and the solid/outer boundary interface. In the present manuscript, these general forms are simplified considerably due to our geometric assumptions. In particular we note that normal interfacial velocities $V_n^{\text{cell/liq}}$ and $V_n^{\text{liq/sol}}$ can be represented by dR_C/dt and dR_I/dt , respectively. The boundary conditions include three at the inner-most boundary ($r = 0$), one at the outer-most boundary ($r = R_O$)

noting that we have neglected species diffusion in the solid, and six at each of the cell/liquid and liquid/ice boundaries ($r = R_C$ and $r = R_I$, respectively). Two additional conditions are required to determine the unknown interface positions, $r = R_C$ and $r = R_I$. Finally, we must have initial conditions for all temperature and concentrations as well as the initial location of the boundaries.

2.2.1. External Wall Boundary Conditions—In this model we assume that the external temperature boundary is modeled by the function $T_{\text{ext}}(t) = T_O$ for $t > 0$ and $T_{\text{ext}}(t) = T_{\mathcal{L}}^0$ for $t = 0$.

2.2.2. Solidification Boundary—At $r = R_I$ we impose the boundary conditions (c.f. Eqs. 25, 30, 31, and the discussion in section 3.6 of Part II of this series)

$$\begin{aligned}\rho_S L \frac{dR_I}{dt} &= k^{\text{sol}} \frac{\partial T_S}{\partial r} - k^{\text{liq}} \frac{\partial T_L}{\partial r}, \\ (A_L - A_S) \frac{dR_I}{dt} &= -D_A^{\text{liq}} \frac{\partial A_L}{\partial r}, \\ (B_L - B_S) \frac{dR_I}{dt} &= -D_B^{\text{liq}} \frac{\partial B_L}{\partial r}, \\ A_S &= k_A A_L, \\ B_S &= k_B B_L, \\ T_S = T_L = T_{\mathcal{L}} &\equiv T_{M0} + T_M^{\text{pres}} + T_M^{\text{os}} + T_M^{\text{GT}},\end{aligned}\tag{4}$$

where L is the latent heat per unit mass, k^{sol} and k^{liq} are the solid and liquid thermal conductivities and k_A and k_B are the segregation coefficients associated with the change of phase between solid and liquid. Finally, $T_{\mathcal{L}}$ represents the freezing temperature whose departure from the equilibrium freezing temperature of pure water T_{M0} is determined by several different effects; T_{M0} is a pressure-dependent correction to the freezing temperature, T_M^{pres} is the freezing point depression influenced by the presence of solutes through the osmolality $\pi = \pi(A_L, B_L)$ and T_M^{GT} is the interface curvature dependence of the interface melting temperature expressed by the Gibbs–Thomson condition [13, 16]. We characterize these effects in more detail next.

Using the pure material derivation of the pressure dependent freezing point depression (see Sec. 3.5.1 in [7]), we take the dependence of the freezing temperature on the pressure p to have the form

$$T_M^{\text{pres}} = T_{M0} \left[-1 + [1 + \beta(p - p_0)] \frac{1}{L\beta} \left(\frac{1}{\rho_L} - \frac{1}{\rho_S} \right) \right],\tag{5}$$

where the solid and liquid densities, ρ_S and ρ_L respectively, are interpreted to be evaluated at the reference pressure p_0 . We have assumed for simplicity that the liquid and solid isothermal compressibilities are the same and equal to β . The pressure is related to the solid and liquid densities and the solidification geometry by

$$p - p_0 = \frac{\rho_L - \rho_S}{\beta \left(\rho_S + \rho_L \frac{V_L}{V_S} \right)} = \frac{\left(\frac{\rho_L}{\rho_S} - 1 \right) \left(1 - \frac{R_I^3}{R_O^3} \right)}{\beta \left[1 + \left(\frac{\rho_L}{\rho_S} - 1 \right) \frac{R_I^3}{R_O^3} \right]}, \quad (6)$$

where we have used the spherical geometry and have included the cell volume as part of the liquid volume in this approximation [see also equation (57) in [7]]. Together these lead to the result

$$T_M^{\text{pres}} = T_{M0} \left\{ -1 + \left[1 - \left(1 - \frac{\rho_S}{\rho_L} \right) \left(1 - \frac{R_I^3}{R_O^3} \right) \right]^{\frac{1}{L\beta\rho_S} \left(1 - \frac{\rho_S}{\rho_L} \right)} \right\} \leq 0. \quad (7)$$

Observe that a difference in densities between solid and liquid phases drives this melting point depression once solidification begins and $R_I < R_O$. As the disparity between these two densities increases due to changing solution densities, and the solid phase grows inward, the melting temperature is lowered thus inhibiting further solidification. This form of the temperature dependence on pressure is similar to the one derived by Conti[15] but takes a slightly different form owing to a constant density assumption used by Conti when integrating the Clapeyron relation. Conti's work, which corresponds to a one-dimensional slab configuration also assumes that the container is elastic whereas we have assumed that the external container is rigid.

Perhaps the simplest model for the solute dependence of the freezing point depression is the linear relation

$$T_M^{\text{os}} = m_A A_L + m_B B_L, \quad (8)$$

where m_A and m_B are liquidus slopes obtained from the equilibrium phase diagram (negative values of m_A and m_B imply that the freezing temperature decreases with increasing concentration). Such models have been used to characterize ternary alloy solidification (e.g. Anderson [2], Anderson and Schulze [3]). For cryobiology applications, more sophisticated models have been developed, for example, by Prickett *et al.* [33] that address non-dilute scenarios. However, discarding the higher order terms in the dilute limit, the model presented by Prickett *et al.* reduces to the form in equation (8) where the liquidus slopes m_A and m_B can be interpreted as

$$m_A = -1.86 \times 10^{-3} \frac{\text{m}^3 \text{K}}{\text{mol}}, \quad m_B = -1.86 \times 10^{-3} k_{\text{diss}} \frac{\text{m}^3 \text{K}}{\text{mol}}, \quad (9)$$

where we have used $M_W = 1.8 \times 10^{-2} \text{ kg mol}^{-1}$ and $V_M = 1.8 \times 10^{-5} \text{ m}^3 \text{ mol}^{-1}$. We anticipate that for quantitative predictions for cryobiological applications more accurate osmolality relations should be used in conjunction with nondilute species diffusion equations. For simplicity in the present work, we consider standard (dilute) diffusion equations and therefore also focus only on the linear relation given in equation (8).

The Gibbs–Thomson term can be expressed as

$$T_M^{\text{GT}} = -T_{\text{MO}}\Gamma\mathcal{K}, \quad (10)$$

where $\mathcal{K}$ is twice the mean curvature of the interface and $\Gamma = \sigma/(\rho_S L)$ is a capillary length scale involving interfacial surface tension σ . For an inward growing spherical solidification front with radius $r = R_I$, $\mathcal{K} = -2R_I^{-1}$, and thus

$$T_M^{\text{GT}} = 2T_{\text{MO}}\Gamma/R_I \geq 0. \quad (11)$$

The terms T_M^{pres} and T_M^{os} in general lower the interface freezing temperature (retard solidification) while T_M^{GT} raises the interface melting temperature (pro-motes solidification). Liu *et al.* [28] have measured the coefficient $T_{\text{MO}}\Gamma$ in water for solidification in glass capillary tubes and found $2T_{\text{MO}}\Gamma = 0.050 \text{ K } \mu\text{m}$ [see their equation (9)]. For $T_{\text{MO}} = 273.15 \text{ K}$ this gives $\Gamma = 9.15 \times 10^{-5} \mu\text{m}$. Note that if the radius of the solidification front is larger than that of the cell (typical cells under consideration have radius $5 \mu\text{m}$ to $50 \mu\text{m}$) the change in melting temperature associated with the Gibbs-Thomson effect is 10^{-2} K or smaller. Further, note that unlike the present situation, the ice shapes reported in Liu *et al.* [28] were convex and accordingly the Gibbs-Thomson effect there corresponded to a freezing point depression (see their Figures 6 and 7).

2.2.3. Cell Membrane—Geometrically, the cell membrane is a region of finite thickness l separating the cell interior from the surrounding liquid. Therefore, in order to describe heat and mass transport between the cell interior and the surrounding liquid one must address transport processes inside the cell membrane. These processes then provide the link between the cell interior and the liquid. Our operating assumption is that the cell membrane has thickness much smaller than the cell radius and much smaller than diffusive length scales (e.g. $l \ll R_C$ and $l \ll 2\sqrt{D\tau}$ where D is the thermal or solute diffusivity in the membrane).

A detailed derivation of the boundary conditions we apply at the cell membrane is given in Part 2 of this series [7]. As shown there, for the dilute limit under consideration we have the following boundary conditions at $r = R_C$

$$\begin{aligned} k^{\text{cell}} \frac{\partial T_C}{\partial r} &= k^{\text{liq}} \frac{\partial T_L}{\partial r}, \\ k^{\text{liq}} \frac{\partial T_L}{\partial r} &= \frac{k^{\text{mem}}}{l} (T_L - T_C), \\ (\Phi_A - 1)A_C \frac{dR_C}{dt} &= D_A^{\text{cell}} \frac{\partial A_C}{\partial r} - \Phi_A P_A^{\text{mem}} (A_L - A_C), \\ -C_C \frac{dR_C}{dt} &= D_C^{\text{cell}} \frac{\partial C_C}{\partial r}, \\ (1 - \Phi_A)A_L \frac{dR_C}{dt} &= \Phi_A P_A^{\text{mem}} (A_L - A_C) - D_A^{\text{liq}} \frac{\partial A_L}{\partial r}, \\ B_L \frac{dR_C}{dt} &= -D_B^{\text{liq}} \frac{\partial B_L}{\partial r}, \end{aligned} \quad (12)$$

where Φ_A is the partition coefficient associated with the cell membrane. That is, $A^{\text{mem}} = \Phi_A A_L$ at the liquid–membrane boundary and $A^{\text{mem}} = \Phi_A A_C$ at the cell–membrane boundary.

We now seek a specific constitutive equation for the cell volume flux. In Part 2 of this series [7] the local volume flux across the cell membrane was shown to be the difference between the local fluid normal velocity and the normal velocity of the cell membrane. We now assume that this volume flux can be expressed in terms of the barycentric mass flux of the transported components, $\mathbf{J}_W^B$ and $\mathbf{J}_A^B$, as

$$J_{\text{vol}} = \frac{1}{\rho_W} \mathbf{J}_W^B \cdot \hat{\mathbf{n}} + \frac{1}{\rho_A} \mathbf{J}_A^B \cdot \hat{\mathbf{n}}, \quad (13)$$

where, from equation (76) in [7] the normal component of the fluxes $\mathbf{J}_i^B \cdot \hat{\mathbf{n}}$ are

$$\mathbf{J}_A^B \cdot \hat{\mathbf{n}} = - \frac{\left\langle (\mathcal{M}_A^B A)_{\text{mem}}^{-1} \right\rangle^{-1}}{l} (\mu_A^{\text{liq}} - \mu_A^{\text{cell}}), \quad (14)$$

$$\mathbf{J}_W^B \cdot \hat{\mathbf{n}} = - \frac{\left\langle (\mathcal{M}_W^B W)_{\text{mem}}^{-1} \right\rangle^{-1}}{l} (\mu_W^{\text{liq}} - \mu_W^{\text{cell}}), \quad (15)$$

$$\mathbf{J}_B^B \cdot \hat{\mathbf{n}} = \mathbf{J}_C^B \cdot \hat{\mathbf{n}} = 0, \quad (16)$$

where l is the membrane thickness, the zero fluxes $\mathbf{J}_B^B$ and $\mathbf{J}_C^B$ are associated with nonpermeating solutes, and where the averaging operator $\langle \cdot \rangle$ and key quantities that use it are discussed in the Part 2 Supplement [8]. Since $\rho_A = A M_A$ and $\rho_W = W M_W$ (see eq. (23) in Part 1 [4]), where M_A and M_W are the molecular mass of solute A and water, respectively, we find that

$$J_{\text{vol}} = - \mathcal{P}_A (\mu_A^{\text{liq}} - \mu_A^{\text{cell}}) - \mathcal{P}_W (\mu_W^{\text{liq}} - \mu_W^{\text{cell}}), \quad (17)$$

Where

$$\mathcal{P}_A \equiv \frac{1}{l M_A} \frac{\left\langle (\mathcal{M}_A^B A)_{\text{mem}}^{-1} \right\rangle^{-1}}{A_{\text{mem}}}, \quad \mathcal{P}_W \equiv \frac{1}{l M_W} \frac{\left\langle (\mathcal{M}_W^B W)_{\text{mem}}^{-1} \right\rangle^{-1}}{W_{\text{mem}}}, \quad (18)$$

are permeability-like coefficients. Note that this form for J_{vol} has the property that $J_{\text{vol}} > 0$ when material (water or CPA) leaves the cell. Recall eq. (80) in [7]:

$$J_{\text{vol}} = (\mathbf{v} - \mathbf{V}_I) \cdot \hat{\mathbf{n}}, \quad (19)$$

and assume that $\mathbf{v} = 0$ in the membrane. Then

$$\frac{dR_C}{dt} = V_n^{\text{mem/cell}} = V_n^{\text{liq/mem}} = V_I \cdot n = -J_{\text{vol}}, \quad (20)$$

indicating that the normal velocity of the membrane, and in this case dR_C/dt , can be written in terms of jumps in chemical potential.

The chemical potentials used by Elliott *et al.* [1, 20] shown in equations (64)–(66) in [4] can be written in terms of concentration variables $c_k = x_k/V_M$, where x_k is the mole fraction of species k [here we have the correspondence $c_1 \rightarrow W$, $c_2 \rightarrow A$ and $c_3 \rightarrow B$ (in the liquid) or $c_3 \rightarrow C$ (in the cell)]. In the liquid we have

$$\begin{aligned} \mu_W^{\text{liq}} &= \mu_W^* + RT \ln[1 - V_M(A_L + B_L)] \\ &\quad + V_M^2[\omega_{WA}A_L(A_L + B_L) + \omega_{WB}B_L(A_L + B_L) - \omega_{AB}A_LB_L], \end{aligned} \quad (21)$$

$$\begin{aligned} \mu_A^{\text{liq}} &= \psi_A + RT \ln(V_MA_L) + \omega_{WA}(1 - V_MA_L)[1 - V_M(A_L + B_L)] \\ &\quad - \omega_{WB}V_MB_L[1 - V_M(A_L + B_L)] + \omega_{AB}V_MB_L(1 - V_MA_L), \end{aligned} \quad (22)$$

and in the cell we have

$$\begin{aligned} \mu_W^{\text{cell}} &= \mu_W^* + RT \ln[1 - V_M(A_C + C_C)] \\ &\quad + V_M^2[\omega_{WA}A_C(A_C + C_C) + \omega_{WC}C_C(A_C + C_C) - \omega_{AC}A_CC_C], \end{aligned} \quad (23)$$

$$\begin{aligned} \mu_A^{\text{cell}} &= \psi_A + RT \ln(V_MA_C) + \omega_{WA}(1 - V_MA_C)[1 - V_M(A_C + C_C)] \\ &\quad - \omega_{WC}V_MC_C[1 - V_M(A_C + C_C)] + \omega_{AC}V_MC_C(1 - V_MA_C). \end{aligned} \quad (24)$$

For simplicity in this manuscript we neglect quadratic terms, yielding

$$\mu_W^{\text{cell}} = \mu_W^* + RT \ln[1 - V_M(A_C + C_C)] + \dots,$$

$$\mu_A^{\text{cell}} = \psi_A + RT \ln(V_MA_C) + \dots,$$

$$\mu_W^{\text{liq}} = \mu_W^* + RT \ln[1 - V_M(A_L + B_L)] + \dots,$$

$$\mu_A^{\text{liq}} = \psi_A + RT \ln(V_MA_L) + \dots$$

Finally, taking differences across the membrane, we find

$$\begin{aligned}
\Delta\mu_A &= \mu_A^{liq} - \mu_A^{cell} = RT \ln(A_L/A_C) + \dots \\
&\approx RT \frac{1}{A_C} (A_L - A_C) + \mathcal{O}(A_L - A_C)^2, \\
&\approx RT \frac{2}{A_C + A_L} (A_L - A_C) + \mathcal{O}(A_L - A_C)^2,
\end{aligned} \tag{25}$$

$$\begin{aligned}
\Delta\mu_W &= \mu_W^{liq} - \mu_W^{cell} = RT \ln \left[\frac{1 - V_M(A_L + B_L)}{1 - V_M(A_C + C_C)} \right] \\
&\approx RT V_M [-A_L - B_L + A_C + C_C] + \dots
\end{aligned} \tag{26}$$

Note that the second form of the approximation in equation (25) remains bounded for the situation in which A_C approaches zero without A_L also approaching zero. This form is also consistent with an analogous term in the “two-parameter (2P)” framework discussed below. Then, combining eqs. (17), (25) and (26), we arrive at the specific cell volume flux equation

$$J_{vol} \approx -P_A^{mem} \frac{2}{A_C + A_L} (A_L - A_C) - P_W^{mem} (A_C - A_L + C_C - B_L), \tag{27}$$

where

$$P_A^{mem} \equiv RT \mathcal{P}_A, \quad P_W^{mem} \equiv RT V_M \mathcal{P}_W. \tag{28}$$

Finally, from eq. (20), we have

$$\frac{dR_C}{dt} = V_n^{cell/liq} = P_A^{mem} \frac{2}{A_C + A_L} (A_L - A_C) + P_W^{mem} (A_C - A_L + C_C - B_L).$$

and we can derive the total cell volume change by integrating over the spherical surface area yielding

$$\frac{dV}{dt} = 4\pi R_C^2 \frac{dR_C}{dt}. \tag{29}$$

This is, of course, the same result one obtains by differentiating the spherical volume $V = \frac{4}{3}\pi R_C^3$ with respect to time. Note that this variable surface area is in line with the body of literature on surface area regulation [21, 31, 36].

Comparison to Existing Volume Flux Models.: It is constructive to make a comparison of the model for volume flux result shown in equation (27) and the resulting formula for dV/dt to existing ones. In the context of classical cryobiological modeling with no spatial gradients, given chemical potential differences in eqs. (25) and (26), we can compare eq. (27) to the volume flux from the Kedem and Katchalsky formalism [24]

$$J_{vol}^{KK} = L_p RT [B_L - C_C + \sigma(A_L - A_C)].$$

where L_p is the hydraulic conductivity and σ is a solute reflection coefficient. Note that in our context all concentrations are evaluated at their respective membrane boundaries and that in the original context these concentrations would reflect “perfect stirring” (i.e. no spatial concentration gradients) and, usually, “infinite bath” (i.e. extracellular spaces are unlimited) assumptions. Additionally, note that we have expressed this volume flux with the same sign convention as our J_{vol} in the sense that a positive volume flux corresponds to material (water or CPA) leaving the cell. The “two-parameter” formalism [25] is obtained by observing that the total volume flux can be partitioned into water volume and solute volume fluxes, similarly to equation eq. (27):

$$J_{vol}^{2P} = J_{vol}^{water} + J_{vol}^{solute} = L_p R T V_M (A_L + B_L - A_C - C_C) - \bar{v}_A P_s \frac{2(A_L - A_C)}{A_C + A_L},$$

where P_s is a solute permeability and we have again chosen a sign convention for volume flux to match our choice in equation (27). Typically, however, the solute permeability is implicitly defined as a constant $\bar{P}_s = 2P_s/(A_C + A_L)$, to arrive at the usual “two-parameter” formalism for volume flux[25],

$$J_{vol}^{2P} = L_p R T (A_L + B_L - A_C - C_C) - \bar{v}_A \bar{P}_s (A_L - A_C).$$

To compare volume fluxes under the model derived here and those from the two-parameter model in its conventional context, we must define these fluxes as functions of the volume averaged intracellular state. To do this, we need a few quantities. In particular, the cell volume, $V = \frac{4}{3}\pi R_C^3$ can be written as $V = V_w + \sum_{i=1}^n \bar{v}_i N_i + V_b$ where V_w is the intracellular water volume, N_i and $\bar{v}_i$ are the moles of the i th intracellular solute and its associated partial molar volume, and V_b is the volume of the cell that is osmotically inactive (see, e.g. [9]).

In the formula for J_{vol}^{2P} in general we should define A_C and C_C as quantities constant throughout the cell (as opposed to pointwise-defined, spatially-dependent quantities). Computations with our present model that treat A_C and C_C as spatially varying throughout the cell in fact often show that the spatial variation in the cell is minor indicating that A_C and C_C effectively represent the average concentrations in the cell [that is, for example, $A_C(r, t) \approx A_C(R_C, t) \approx A_C^{ave}(t)$]. Interpreting A_C and C_C in this way then allows us to write

$$A_C = N_A / (V_w + \bar{v}_A N_A + \bar{v}_C N_C), \quad (30)$$

$$C_C = N_C / (V_w + \bar{v}_A N_A + \bar{v}_C N_C). \quad (31)$$

The fluxes J_{vol}^{water} , J_{vol}^{solute} are then used independently to determine the intracellular state:

$$\begin{aligned}\frac{dV_w}{dt} &= -J_{\text{volw}}^{\text{water}} \mathcal{A} = -L_p \mathcal{A} RT (A_L + B_L - A_C - C_C), \\ \bar{v}_A \frac{dN_A}{dt} &= -J_{\text{vol}}^{\text{solute}} \mathcal{A} = P_s \mathcal{A} (A_L - A_C),\end{aligned}\quad (32)$$

where $\mathcal{A}$ is cell membrane surface area. The membrane surface area is often assumed fixed at $\mathcal{A} = 4\pi(R_C^{\text{iso}})^2$ where R_C^{iso} is the cell radius under isosmotic extracellular conditions. This is not the assumption we make in our model—we have written $\mathcal{A} = 4\pi R_C^2$ which is a time-dependent quantity—see equation (29). With the fixed surface area assumption one obtains a modified relationship between dR_C/dt and J_{vol} as follows:

$$\frac{1}{\mathcal{A}} \frac{dV}{dt} = \frac{1}{\mathcal{A}} \frac{4\pi}{3} \frac{dR_C^3}{dt} = \frac{4\pi R_C^2}{4\pi R_C^{\text{iso}2}} \frac{dR_C}{dt} = \left(\frac{R_C}{R_C^{\text{iso}}}\right)^2 \frac{dR_C}{dt} = -J_{\text{vol}}.$$

Compared with equation (20), we note the additional term accounting for the relative change of surface area over the isosmotic surface area. Thus, we have

$$\begin{aligned}\frac{dR_C}{dt} &= -\left(\frac{R_C^{\text{iso}}}{R_C}\right)^2 J_{\text{vol}}, \\ &= -\left(\frac{R_C^{\text{iso}}}{R_C}\right)^2 (J_{\text{volw}}^{\text{water}} + J_{\text{vol}}^{\text{solute}}), \\ &= \left(\frac{R_C^{\text{iso}}}{R_C}\right)^2 \mathcal{A}^{-1} \left(\frac{dV_w}{dt} + \bar{v}_A \frac{dN_A}{dt}\right), \\ &= \left(\frac{R_C^{\text{iso}}}{R_C}\right)^2 [-L_p RT (A_L + B_L - A_C - C_C) + \bar{v}_A P_s (A_L - A_C)].\end{aligned}\quad (33)$$

To proceed with our comparison, we will assume that extracellular concentration A_L and B_L are known, either through our modeling or by assumption of perfect stirring. We may calculate A_C and C_C using ODE system (32) coupled with equations (30) and (31) and initial conditions

$$V_w(0) = V_w^{\text{init}}, \quad (34)$$

$$N_A(0) = \frac{V_w^{\text{init}} A_C(0)}{1 - \bar{v}_A A_C(0) - \bar{v}_C C_C(0)}. \quad (35)$$

Finally, note that this presentation differs from much of the published work using the two-parameter model in that we use concentration instead of molality to describe the chemical potential difference across the cell membrane. To obtain a similar model using the molality form, we could use an appropriate chemical potential form from the supplement of Part I [5],

make similar arguments about the higher order terms, and observe that the intracellular molality of solute A is defined by $m_A = N_A / \rho_w V_w$, to arrive at

$$\begin{aligned} \frac{dV_w}{dt} &= -J_{\text{vol}_w}^{\text{water}} \mathcal{A} = -L_p \mathcal{A} RT \left(m_A^L + m_B^L - \frac{N_A + N_C}{\rho_w V_w} \right), \\ \bar{v}_s \frac{dN_A}{dt} &= -J_{\text{vol}}^{\text{solute}} \mathcal{A} = P_s \mathcal{A} \left(m_A^L - \frac{N_A}{\rho_w V_w} \right), \end{aligned} \quad (36)$$

where m_A^L and m_B^L are the extracellular molalities of solutes A and B , respectively (see Benson [10] for a thorough discussion of this in the context of molality).

2.2.4. Initial Conditions—Finally, the initial conditions for this model are $R_I = R_O$, $R_C = R_C^{\text{init}}$. Unless otherwise noted we shall assume that the initial temperature and concentrations in the liquid and cell satisfy

$$T^{\text{init}} = m_A A_L^{\text{init}} + m_B B_L^{\text{init}} = m_A A_C^{\text{init}} + m_C C_C^{\text{init}}, \quad (37)$$

where m_C is a liquidus slope associated with the intercellular non-permeating solute C . Generally, we shall assume that A_L^{init} and B_L^{init} are specified as independent parameters. We then assume that $A_C^{\text{init}} = A_L^{\text{init}}$ and that C_C^{init} is chosen to satisfy the second equality, though in practice, R_C^{init} and C_C^{init} are related at equilibrium via the Boyle van't Hoff relationship (c.f. [9]).

3. Nondimensionalization

We introduce dimensionless variables denoted by primes

$$r' = \frac{r}{R_O}, t' = \frac{t}{(R_O^2 / \kappa^{\text{sol}})}, T' = \frac{T - T_{M0}}{T_{M0} - T_O}, A' = \frac{A}{A_R}, B' = \frac{B}{B_R}, \text{ and } C' = \frac{C}{C_R}, \quad (38)$$

where A_R , B_R and C_R are constant reference values of these concentration variables. For consistency with the previous two parts, we shall assume that $A_R = B_R = C_R = V_M^{-1}$ where $V_M = 0.018$ L/mol.

The above forms for temperature and concentration are applied in all regions. While in practice in this cryobiological setting, with orders-of-magnitude temperature and concentration changes, many if not all of the key parameters of this model vary with both temperature and concentration (and even pressure), here we assume that all material parameters are constant.

3.1. Bulk phases

In the nondimensionalized variables, systems (1)–(3) become

$$\left. \begin{aligned} \frac{\partial T'_S}{\partial t'} &= \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial T'_S}{\partial r'} \right), \\ \frac{\partial A'_S}{\partial t'} &= 0, \\ \frac{\partial B'_S}{\partial t'} &= 0. \end{aligned} \right\} \quad R'_I < r' < 1 \quad (39)$$

$$\left. \begin{aligned} \frac{\partial T'_L}{\partial t'} &= \kappa_{ls} \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial T'_L}{\partial r'} \right), \\ \frac{\partial A'_L}{\partial t'} &= \frac{1}{Le_A^{\text{liq}}} \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial A'_L}{\partial r'} \right), \\ \frac{\partial B'_L}{\partial t'} &= \frac{1}{Le_B^{\text{liq}}} \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial B'_L}{\partial r'} \right), \end{aligned} \right\} \quad R'_C < r' < R'_I \quad (40)$$

$$\left. \begin{aligned} \frac{\partial T'_C}{\partial t'} &= \kappa_{cs} \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial T'_C}{\partial r'} \right), \\ \frac{\partial A'_C}{\partial t'} &= \frac{1}{Le_A^{\text{cell}}} \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial A'_C}{\partial r'} \right), \\ \frac{\partial C'_C}{\partial t'} &= \frac{1}{Le_C^{\text{cell}}} \frac{1}{r'^2} \frac{\partial}{\partial r'} \left(r'^2 \frac{\partial C'_C}{\partial r'} \right), \end{aligned} \right\} \quad 0 < r' < R'_C \quad (41)$$

where $\kappa_{ls} = \kappa^{\text{liq}}/\kappa^{\text{sol}}$, $\kappa_{cs} = \kappa^{\text{cell}}/\kappa^{\text{sol}}$, and boundedness conditions apply on the temperature and concentration variables at $r' = 0$.

3.2. Interfacial/boundary and initial conditions

At $r' = 1$ we have

$$T'_S = f(r'),$$

Where

$$f(r') = \frac{T_{\text{ext}}(t) - T_{M0}}{T_{M0} - T_O} = [T'_{\mathcal{L}^0} + 1]\gamma(r') - 1, \quad (42)$$

where $T'_{\mathcal{L}^0}$ is the dimensionless initial value of the freezing temperature defined below in equation (51) and $\gamma(r')$ is the dimensionless external temperature function.

At $r' = R'_I$ we have

$$S \frac{dR'_I}{dt'} = \frac{\partial T'_S}{\partial r'} - \kappa_{ls} \frac{\partial T'_L}{\partial r'}, \quad (43)$$

$$(A'_L - A'_S) \frac{dR'_I}{dt'} = - \frac{1}{Le_A^{\text{liq}}} \frac{\partial A'_L}{\partial r'}, \quad (44)$$

$$(B'_L - B'_S) \frac{dR'_I}{dt'} = - \frac{1}{Le_B^{\text{liq}}} \frac{\partial B'_L}{\partial r'}, \quad (45)$$

$$A'_S = k_A A'_L, \quad (46)$$

$$B'_S = k_B B'_L, \quad (47)$$

$$T'_S = T'_L = T'_{\mathcal{L}} = E(R'_I) + \bar{m}_A A'_L + \bar{m}_B B'_L + \frac{2\bar{\Gamma}}{1-\tau} \frac{1}{R'_I}, \quad (48)$$

where

$$E(R'_I) = \frac{1}{1-\tau} \left\{ -1 + \left[1 - \rho(1 - R'^3_I) \right]^{\rho' \bar{\beta}} \right\}. \quad (49)$$

and

$$S = \frac{L}{c_{ps}(T_{M0} - T_O)}, \quad k_{ls} = \frac{k^{\text{liq}}}{k^{\text{sol}}}, \quad Le_A^{\text{liq}} = \frac{\kappa^{\text{sol}}}{D_A^{\text{liq}}}, \quad Le_B^{\text{liq}} = \frac{\kappa^{\text{sol}}}{D_B^{\text{liq}}}, \quad \tau = \frac{T_O}{T_{M0}}, \quad (50)$$

$$\bar{m}_A = \frac{m_A A_R}{T_{M0} - T_O}, \quad \bar{m}_B = \frac{m_B B_R}{T_{M0} - T_O}, \quad \bar{\beta} = \rho_S L \beta, \quad \rho = 1 - \frac{\rho_S}{\rho_L}, \quad \bar{\Gamma} = \frac{\Gamma}{R_O}.$$

The corresponding initial value of the dimensionless freezing temperature is 336 given by

$$T'_{\mathcal{L}0} = \bar{m}_A A'^{\text{nit}} + \bar{m}_B B'^{\text{nit}} + \frac{2\bar{\Gamma}}{1-\tau}, \quad (51)$$

where we note the pressure contribution to the freezing temperature does not contribute [i.e. $E(R'_I = 1) = 0$ in equation (49)]. As mentioned above for this value of the radius, the Gibbs-Thomson term is technically nonzero, but is 340 numerically small and can be neglected in the calculation of $T'_{\mathcal{L}0}$.

At $r' = R'_C$ we have

$$\frac{\partial T'_C}{\partial r'} = k_{lc} \frac{\partial T'_L}{\partial r'},$$

$$\epsilon k_{lm} \frac{\partial T'_L}{\partial r'} = T'_L - T'_C,$$

$$(\Phi_A - 1)A'_C \frac{dR'_C}{dt'} = \frac{1}{Le_A^{\text{cell}}} \frac{\partial A'_C}{\partial r'} - \Phi_A \overline{P_A^{\text{mem}}} (A'_L - A'_C),$$

$$-C'_C \frac{dR'_C}{dt'} = \frac{1}{Le_C^{\text{cell}}} \frac{\partial C'_C}{\partial r'},$$

$$(1 - \Phi_A)A'_L \frac{dR'_C}{dt'} = \Phi_A \overline{P_A^{\text{mem}}} (A'_L - A'_C) - \frac{1}{Le_A^{\text{liq}}} \frac{\partial A'_L}{\partial r'},$$

$$B'_L \frac{dR'_C}{dt'} = - \frac{1}{Le_B^{\text{liq}}} \frac{\partial B'_L}{\partial r'},$$

$$\frac{dR'_C}{dt'} = \overline{P_A^{\text{mem}}} \frac{2}{A'_C + A'_L} (A'_L - A'_C) + \overline{P_W^{\text{mem}}} (A'_C - A'_L + C'_C - B'_L),$$

where

$$k_{lc} = \frac{k^{\text{liq}}}{k^{\text{cell}}}, \quad k_{lm} = \frac{k^{\text{liq}}}{k^{\text{mem}}}, \quad Le_A^{\text{cell}} = \frac{\kappa^{\text{sol}}}{D_A^{\text{cell}}}, \quad Le_C^{\text{cell}} = \frac{\kappa^{\text{sol}}}{D_C^{\text{cell}}},$$

$$\epsilon = \frac{l}{R_O}, \quad \overline{P_A^{\text{mem}}} = \frac{P_A^{\text{mem}} R_O}{\kappa^{\text{sol}}}, \quad \overline{P_W^{\text{mem}}} = \frac{P_W^{\text{mem}} R_O A_R}{\kappa^{\text{sol}}}.$$

Again, it is possible to include a temperature dependence in the permeability function of the form

$$\overline{P_W^{\text{mem}}} = \overline{P_W^{\text{mem}}} \exp \left[-\mathcal{E} \left(\frac{1}{1 + T(1 - \tau)} - 1 \right) \right], \quad (52)$$

where $\mathcal{E} = E_d/(RT_{\text{MO}})$ is a dimensionless activation energy (Thirumala and Devireddy [37]). Setting $\mathcal{E} = 0$ recovers a simple model in which the permeability is taken to be the constant value $\overline{P_W^{\text{mem}}}$, although as noted in Table 2 the estimated value for $\mathcal{E}$ based on Thirumala and Devireddy is in the range 25 to 150 [37].

The initial conditions for this problem have $R'_I = 1$, $R'_C = R_C^{\text{init}} = R_C^{\text{init}}/R_O = 0.05$. Unless otherwise noted we shall assume that the initial temperature and concentrations in the liquid and cell satisfy

$$T'^{\text{init}} = \bar{m}_A A_L^{\text{init}} + \bar{m}_B B_L^{\text{init}} = \bar{m}_A A_C^{\text{init}} + \bar{m}_C C_C^{\text{init}}, \quad (53)$$

where $\bar{m}_C = (m_C C_R)/(T_{M0} - T_O)$. As noted in the previous section, we shall assume that $A_C^{\text{init}} = A_L^{\text{init}}$ and that C_C^{init} is chosen to satisfy the second equality. Notice that under the additional assumption of equal liquidus slopes for solutes B and C then $C_C^{\text{init}} = B_L^{\text{init}}$.

3.2.1. Nondimensionalization of the 2P model—Here our goal is to relate our PDE model with the classical model. First, we define the whole-cell isosmotic volume $V^{\text{iso}} = \frac{4}{3}\pi R_C^{\text{iso}3}$, and with total volume $V = V_w + \bar{v}_A N_A + \bar{v}_C N_C + V_b$, we note that in isosmotic conditions $V^{\text{iso}} = V_w^{\text{iso}} + \bar{v}_C N_C + V_b \approx V_w^{\text{iso}} + V_b$. Then, we define $V_w^{\text{iso}} = V^{\text{iso}} - V_b$, and introduce dimensionless volumes $V' = V/V_w^{\text{iso}}$, $V'_w = V_w/V_w^{\text{iso}}$ and $V'_b = V_b/V_w^{\text{iso}}$. The number of moles of permeating solute is $N_A = A_C(V - V_b) = A_R V_w^{\text{iso}} A'_C(V' - V'_b)$. Therefore, a natural dimensionless version of this quantity is $N'_A = N_A/(A_R V_w^{\text{iso}}) = A'_C(V' - V'_b)$. Then the ODE system (32) can be written as

$$\begin{aligned} V_w^{\text{iso}} \frac{dV'_w}{dt} &= -L_p \mathcal{A} RT C_R (A'_L + B'_L - A'_C - C'_C), \\ A_R V_w^{\text{iso}} \frac{dN'_A}{dt} &= \frac{P_s \mathcal{A} C_R}{\bar{v}_A} (A'_L - A'_C), \end{aligned} \quad (54)$$

with auxiliary equations

$$\begin{aligned} A'_C &= N'_A/(V' - V'_b), \\ &= N'_A/(V'_w + \bar{v}_A N'_A/V_w^{\text{iso}} + \bar{v}_C N_C/V_w^{\text{iso}}), \\ &= N'_A/(V'_w + \bar{v}_A N'_A A_R V_w^{\text{iso}}/V_w^{\text{iso}} + \bar{v}_C N'_C A_R V_w^{\text{iso}}/V_w^{\text{iso}}), \\ &= N'_A/(V'_w + \bar{v}_A N'_A A_R + \bar{v}_C N'_C A_R), \end{aligned} \quad (55)$$

and similarly

$$C'_C = N'_C/(V'_w + \bar{v}_A N'_A A_R + \bar{v}_C N'_C A_R). \quad (56)$$

Combining equations (54), (55), and (56) we have

$$\begin{aligned} \frac{dV'_w}{dt} &= -\frac{L_p A R T C_R}{V_w^{\text{iso}}} \left(A'_L + B'_L - \frac{N'_A + N'_C}{V'_w + \bar{v}_A N'_A A_R + \bar{v}_C N'_C A_R} \right), \\ \frac{dN'_A}{dt} &= \frac{P_s A}{V_w^{\text{iso}} \bar{v}_s} \left(A'_L - \frac{N'_A}{V'_w + \bar{v}_A N'_A A_R + \bar{v}_C N'_C A_R} \right). \end{aligned} \quad (57)$$

Finally, we have initial conditions

$$V'_w(0) = \frac{V_w(0)}{V_w^{\text{iso}}} = \frac{V_w^{\text{init}}}{V_w^{\text{iso}}}, \quad (58)$$

$$N'_A(0) = \frac{N_A(0)}{A_R V_w^{\text{iso}}} = \frac{V_w^{\text{init}} A_C(0)}{1 - \bar{v}_A A_C(0) - \bar{v}_C C_C(0)} \frac{1}{A_R V_w^{\text{iso}}}. \quad (59)$$

4. Determination of the permeability coefficients $\overline{P_A^{\text{mem}}}$ and $\overline{P_W^{\text{mem}}}$

While many of the parameters of the present model are known independently of this biological setting, the lumped membrane permeability to water and so374 lutes in the context of our model is not known. Therefore, we fit our model to predictions from an ordinary differential equation membrane transport model (the “2P” model, see [26] for a review) with parameters known for a B6 mouse embryonic stem cell (mESC) line [23]. There are considerable challenges in estimating the correct parameters for a spatially dependent (PDE) model given a prediction from a non-spatially dependent (ODE) model. In particular, there may be information not captured when fitting the ODE model to experimental data. For example, in our case, measurements of the permeability under the ODE model regime of mESC were made at temperatures between 273.15 K and 310.15 K, (0 °C and 37 °C) and the present numerical experiments were made at 258.15 K (−15 °C). The Arrhenius equation used by Kashuba Benson et al., was used to extrapolate down to 233.15 K, and while this extrapolation model proved successful for Kashuba Benson et al. in predicting more-optimal cooling protocols for mouse embryonic stem cells, there have been a number of studies demonstrating that extrapolating mass transport kinetic parameters to low temperatures is inaccurate [17–19]. Moreover, the effects of spatial gradients generated by solute polarization at both the ice and cell boundaries are only accounted for as lumped parameters in ODE models, and as such would not reflect the full dynamics of the system. Thus, in the absence of actual experimental data describing the dynamic state of the system, and in particular, the location of the ice and cell boundaries, we must settle for a reasonable approximation of the true dynamics.

To find $\overline{P_A^{\text{mem}}}$ and $\overline{P_W^{\text{mem}}}$, we used the nondimensional form of the 2P System (57) with $A_L(t)$ and $B_L(t)$ given by the boundary concentrations of the solution of the PDE system. That is, given a set of parameters $\overline{P_A^{\text{mem}}}$ and $\overline{P_W^{\text{mem}}}$, the PDE system was solved and the nondimensional forms of the time-dependent concentrations $A_L(t)$ and $B_L(t)$ were determined. Then the nondimensional version of the 2P system (57) was solved using these extracellular concentrations and the sum of squared error between the predicted radius for both models at 100 evenly distributed time points was minimized using Matlab’s (Natick, Massachusetts: The MathWorks Inc.) built in “fmincon” constrained minimization algorithm.¹ Best fits of the data at four temperatures are shown in figure 2, and the

¹Certain commercial equipment, instruments, or materials are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

temperature dependence of the water and solute permeability values from our model are shown in Figure 3.

5. Results and Discussion

There are myriad effects that can be explored with the somewhat simplified model presented in the manuscript. After exploring the basic behavior of our system, we will restrict our analysis to the following two effects that are not usually accounted for in cryobiological modeling: 1) the effect of ice-liquid density changes that increase pressure related freezing point depression at the ice-liquid interface 2) the effect of non-zero partition coefficients. The numerical procedure used to compute the solutions to our model combines a finite difference discretization of the spatial derivatives, a domain mapping procedure and a method of lines approach for solving a large system of coupled ODEs. This numerical procedure is described in detail in another paper [6]. Unless otherwise noted, the parameter values used are those listed in Table 2.

5.1. Determination of water and solute permeabilities

The 2P model extrapolated to lower temperatures from Kashuba Benson can be well-fit with the model proposed in this study. However, because the 2P model parameters are governed by the Arrhenius relationship, one would expect adherence in the proposed model as well. In fact, The governing activation energies of the 2P model in Kashuba Benson are 5.979×10^4 J/mol and 6.473×10^4 J/mol for L_p and P_s respectively. And the best fit activation energies for the proposed model are $\overline{P_W^{mem}} = 6.4 \times 10^4$ J/mol and $\overline{P_A^{mem}} = 4.1 \times 10^4$ J/mol. To test this further we plot the response of the proposed model with $\overline{P_A^{mem}}$ and $\overline{P_W^{mem}}$ set to the values predicted by the Arrhenius relationship in Fig. 3. One possible explanation for this discrepancy is that there is an explicit concentration dependence of the effective permeability that multiplies the transmembrane concentration differences governing dR_C/dt (see section 2.2.3). Specifically, as temperatures are reduced in our model, the concentrations A_L and B_L increase after the initial ice growth (see Fig. 4). In Eq. (27), J_{vol} appears with $\overline{P_A^{mem}}$ divided by the mean solute concentration A across the membrane. Given an “effective” membrane permeability defined by the 2P model, as this mean A increases with decreasing temperature, a proportionate increase in $\overline{P_A^{mem}}$ and $\overline{P_W^{mem}}$, is required.

5.2. Solution Regimes and Dynamics

We first outline results for the case in which there is complete solute rejection ($k_A = k_B = 0$) and for which the pressure influence on the freezing point is absent ($\rho = 0$). Before 10^{-3} seconds, there is an early time similarity solution associated with the freezing front moving inward from the cold boundary. This is effectively a Stefan problem for the temperature and compositions in the solid and liquid phases. The details of this similarity solution are described in [6]. Here the time scales are small enough that thermal and solutal diffusion effects occurring near the solidification front are not affected by the presence of the cell. The cell remains in its initial state – mass transfer across the cell membrane is negligible on this time scale. Further, the dynamics of the solidification front are independent of the confined

geometry of the problem and locally the solidification proceeds as if the solid/liquid interface was planar.

In the second regime, approximately 10^{-3} to 10^{-1} seconds, the solidification front dynamics are still unaffected by the presence of the cell but the solidification rate is influenced by the finite size effects which leads to a build up of solute (A_L and B_L) in the liquid due to solute rejection ($k_A = k_B = 0$ in the simulation in Figure 4, but similar effects are evident for other values of k_A and k_B , data not shown) at the solid–liquid interface. As a result of the increased solute concentration in the liquid, the corresponding freezing temperature is depressed. Note that in general the melting temperature is influenced by pressure and curvature effects as well, phenomena we explore below. An equilibrium state is reached at approximately 10^{-2} seconds, and inward solidification is arrested when the geometry-induced solute buildup lowers the freezing temperature to the external temperature $f(0)$. Again, these dynamics occur quickly and so no appreciable changes in the size or composition of the cell occur. By the end of this regime, however, solute differences between the liquid and cell have developed. The spatial dependence of the temperature and concentration present at the early times associated with regimes 1 and 2 has more or less been eliminated by the end of the second regime. The absence of spatial variations in the third regime described below suggest that the long time behavior may be well-characterized by simpler ODE models in which temperature and concentrations vary only in time. It is important to note, however, that the concentrations surrounding the cell leading into regime 3 are not the ones given at $t = 0$ but rather those established at the end of the second regime as determined by the full PDE model.

The next regime begins after the initial solidification occurs. Here the solidification front has equilibrated ($dR_I/dt \approx 0$), and A_L and B_L have effectively reached their final equilibrium values and are uniform in space. However, differences in CPA and water concentrations at the cell membrane that have developed during the previous regimes now drive mass transport across the cell membrane on a much longer time scale. The intracellular cell concentration levels, plotted in Figures 4E and 4F, show that there are no discernible intracellular concentration gradients, and because of this R_C behaves as we would expect in the strictly ODE derived case. This is not surprising as the cell radius in this model is a biologically relevant $5 \mu\text{m}$. The characteristic diffusion time ($t_{\text{diff}} = (R/2\sqrt{D})^2$) for DMSO in a room-temperature cell of radius $R = 5 \mu\text{m}$ is about 5×10^{-3} s. Following the Arrhenius law for solute diffusivity and diffusivity activation energy of 2.28×10^4 J/K mol, the characteristic diffusion time at -25°C will still be on the order of 10^{-1} seconds. Because the membrane water and solute diffusivity is more than an order of magnitude lower than the bulk solution, the cell radius changes on the minute time scale, rendering intracellular diffusion modeling relatively unimportant. In short, starting near 1 second, there is an initial transfer of CPA into the cell from the liquid at the same time that water is lost from the cell. Both of these are in response to the elevated salt/CPA concentration in the liquid owing to the solidification-induced solute rejection, continuing until the concentrations and cell radius approach final equilibrium values.

5.3. Pressure induced effects

One area where our model is different from many in the cryobiological literature is the inclusion of pressure effects on melting temperature. While these effects have been explored by Rubinski et al [35] and Conti [15], in the former, individual cell dynamics were not explored, and in the latter, multicomponent transport was not investigated. There has been much recent attention to the use of pressure as an additional control parameter during cryopreservation. In particular it has been suggested as a possible approach for high subzero tissue and organ preservation [38] and food preservation [12, 29]. However, no mathematical model relating these characteristics in individual cells has been proposed.

For the current model, isochoric, or other pressure dependent tissue cryopreservation protocols, it is desirable that the ice is formed external to the cell or tissue (e.g. at the freezing vessel wall away from the tissue). In this case, our model remains relevant even in the scale of tissues, except that in place of the “cell” region Ω_C , the model would have a “tissue” region. In fact, flux into and out of this tissue region as well as transport in the tissue are modeled very similarly to that presented here. For example Benson et al [11] couple transport at the tissue boundary to the extracellular space through a Robin-type (Type III) boundary condition for the intra-tissue ODE-PDE system governing solute movement, and it is common to use the same diffusion model for a tissue interior used in our treatment here. Therefore, we expect that this model approach may be useful for expansion into more complex biological domains than presented here, with particular relevance to partial domain freezing or the use of pressure to minimize intracellular ice formation.

Our treatment in Part II of this series is quite general, allowing for the determination of the effects due to the individual components of the solution. Even though we presented an explicit ternary model in Part II, here we simplified to the water-only form, given in equation (7) dependent on the radius and relative densities of solid and liquid phases. Because we assume pressure is not spatially dependent, and only a function of the relative radius of the ice front, it too could in theory be well-modeled by a system of ordinary differential equations. However, we show in Figure 5 that the solid–liquid interface is significantly slowed with even a small difference in the solid and liquid densities, and there is a significant difference in equilibrium ice radius. This change has major effects on the equilibrium CPA concentration for a given ρ , an effect seen in 6 where the relationship between the ice radius and the CPA concentration at a given expansion coefficient is shown. Here we have modeled up to $\rho = 0.13$, noting that for pure water/ice, $\rho = .0831$, and for 4 molar DMSO, we expect $0.12 \leq \rho \leq 0.134$ [34].

Note that at room temperature, the density of CPA solutions increases with concentration [39], but the thermal expansion coefficient,

$$\alpha = \frac{d \ln \rho}{dT}$$

decreases with concentration [40]. Therefore there is a complicated interplay between expansion, density and pressure that is not perfectly captured here. Further refinement in our

model, then, can be achieved by detailed concentration and temperature dependence of density of the ternary solutions.

5.4. Partition coefficient effects

When one considers the effects of nonzero partition coefficients at the solid–liquid interface it is essential to resolve the spatial dependence in the solute fields. In this setting the solute gradients at the solid–liquid interface and the interface velocity depend on the value of the partition coefficients as well as on the solute diffusivities. An added complexity, which we do not address here, is that the velocity of the ice front affects the partition coefficients [22], so careful understanding of dR_I/dt is needed. The amount of solute incorporated into the solid, and therefore not left behind in the liquid, is a dynamic quantity. Spatially homogenous ODE models cannot capture this because of the coupling between solute rejection, diffusion away from the interface, and the interfacial velocity. Figure 7 shows the differential cell volume responses as a function of solute partition coefficients when the solute diffusivities of the two components are equal. In particular, in Figure 7 there are a few things to note: first is that setting the partition coefficients for both solutes equal to each other results in the same equilibrium concentrations, and these in turn lead to the same cell equilibrium volume. In this context, by equilibrium, we refer to the configuration established after the solid–liquid interface motion has stopped but before appreciable trans-membrane transport has occurred. Note that the equilibrium concentration is a function of the osmolality which in turn is proportional to the freezing point depression. If the partition coefficients are equal, and the solute diffusivities matched (i.e. $D_A^{Li q} = D_B^{Li q}$), then the ratio of solute molarities remains constant—the same proportions of each solute and water are taken up by the solid. To wit, in our model, let the ratio of initial concentrations in the liquid portion be $r = A/B$. Then we can write

$$-T_{FPD}/1.86 = \pi(A, B) = \pi(rB, B)$$

In our present model, π is simplified in the dilute limit to $\pi(A, B) = A + k_{\text{diss}}B$ (c.f. equations (8) and (9)), so $\pi(rB, B) = B(r + k_{\text{diss}})$. Therefore, the final concentrations of A and B (with $A = rB$) are determined entirely by the equilibrium temperature of the system. Therefore the final concentration, and thus the final cell volume will be unaffected in these cases. In contrast, if $k_A \neq k_B$, (and/or if $D_A^{Li q} \neq D_B^{Li q}$) the ratio r will necessarily be non-constant and the equilibrium cell volume will be affected (see Figure 7). This does not mean that the equilibrium ice radius at any given temperature will be the same. In fact, because of the above arguments, if k_A or k_B are nonzero, the equilibrium volume of the liquid region will be affected, as can be seen in Figures 8 and 9.

In Figures 8 and 9 we have also shown the CPA concentration measured in the extracellular region at the cell membrane. It is clear from these curves that the extracellular chemical environment depends in a non-trivial way on the level of solute rejection that occurs at the solidification front. In particular, note the CPA concentration at the cell membrane generally increases as the amount of solute rejection decreases for the cases shown with $k_A = k_B$. That is, when more solute is incorporated into the solid, the solid–liquid interface moves faster

and consequently, the extracellular CPA concentration at the cell membrane is higher at any given point in time. Also shown for further comparison are cases for which the solute rejection coefficients are not equal ($k_A \neq k_B$). Here the solidification front speed is also modified in a nontrivial way.

6. Conclusions

We have presented a model of a 10 μm diameter cell in a 200 μm diameter spherical region undergoing cooling to subzero temperatures in the presence of an encroaching solidification front that includes a number of phenomena that are often neglected in cryobiological modeling. As new examples in application to the prediction of the cellular response to freezing, we have presented results that address the influence of the pressure-dependence of the ice-front freezing temperature and the influence of nonzero solute partition coefficients associated with the solid–liquid interface.

We have found in our simulations that there are always thermal and solutal spatial gradients at early times in the dynamics associated with rapid motion of the ice front. Such gradients are expected to be enhanced for protocols involving increased cooling rates and spatial variations are expected to persist longer in larger systems. For our model of the cell, while these spatial gradients are generally located away from the cell membrane itself, they influence the extracellular environment, and hence the cell dynamics, in nontrivial

On the one hand, our simulations show that for the cooling of a 10 μm diameter cell, it is a good approximation to neglect thermal and solute gradients over time scales of interest for the selective transport of solute across the cell membrane. That is, spatial gradients of temperature and solute in the extracellular liquid dissipate before significant transport across the cell membrane occurs and it would be reasonable to characterize extracellular temperatures and concentrations by spatially-uniform values. On the other hand, the cell dynamics are crucially dependent on the particular values of such spatially-uniform concentrations in the extra-cellular environment. These values depend nontrivially on the motion of the ice front, and are sensitive to even small levels of partial solute rejection at the solidification front. Additionally, these values depend non-trivially on differences between the levels of solute rejection of the solution components, and also differences between the rates of solute diffusion. That is, the actual values of the extracellular concentrations depend on nontrivial interactions between spatial gradients at the ice front, solute rejection, solute diffusion, and the ice front motion. For the case of complete solute rejection at the solidification front, at least as a function of the solid–liquid interface position, a simple solute conservation argument would provide a prediction of a spatially-uniform extracellular concentration. However, predicting such an extracellular concentration as a function of time requires knowledge of the ice front dynamics as a function of time. This latter prediction depends on the external thermal controls. When there is partial solute rejection, the speed of the ice front, and hence the extracellular chemical environment, depends nontrivially on the level of solute rejection, including any disparity of solute rejection between different solutes. Thus, in order to accurately predict the extracellular chemical environment in the presence of an encroaching solidification front, one must predict the ice front dynamics and

understand how these dynamics depend on spatial thermal and solute gradients generated at the ice front.

We have also shown that the ice-front dynamics are strongly dependent on a density/pressure effect. Any model that neglects thermal and solutal gradients must still carefully resolve the solidification front dynamics in order to properly represent the extracellular environment. Certainly, an accurate prediction of the intracellular dynamics requires an accurate characterization of the extracellular environment.

Differences in prediction of the intracellular state that arise due to different characterizations of the extracellular environment (e.g. based on inclusion and/or exclusion of solute rejection effects and/or pressure dependence of freezing temperature) during cooling may explain differences between theoretically predicted damage and observed damage during cooling protocols. Our comparisons of cell volume plots from the constant cooling rate numerical experiments suggest that there are significant enough differences to warrant careful numerical optimization of the combined system followed by experimental testing of newly predicted optimal protocols. Additionally, the implication of the temperature dependence of many thermo-chemical parameters over the wide range of temperatures of relevance for cryobiology is of significant concern. While we have not specifically addressed these thermal effects in the present work, our model presented here provides a foundation upon which such issues can be carefully examined.

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Figure 1:

Regions corresponding to the cellular, liquid, and solid phases under consideration in two schematic forms. The top schematic indicates the general geometry of our spherically symmetric system, and the bottom schematic indicates concentration variables and the movement of their constitutive species across interfaces. In this model R_C , R_I are time dependent and R_O is fixed.

Figure 2:

Best fits of the 2P model with parameters determined or extrapolated from Kashuba Benson et al [23]. R_C/R_C^0 is plotted in the solid line and values of the normalized cell radius from the Kashuba Benson model are given as dots.

Figure 3: Temperature dependence of water and CPA permeabilities fit to the 2P model (dots) at six temperatures. Regression lines indicate log-transformed linear regressions of the Arrhenius model for $\overline{P}_W^{\text{mem}}$ (top) and $\overline{P}_A^{\text{mem}}$ (bottom).

Figure 4:

Plots of a numerical simulation of the system beginning at equilibrium in 1 mol/L DMSO and isosmotic NaCL when the temperature is abruptly changed to -15°C . In this simulation we ignore the freezing point depression and volume effects caused by the difference in solid and liquid densities. We also ignore any effects due to non-complete solute rejection at the ice front. The logarithmic time scale shows clearly the three different time scales for the three transport processes: thermal, solute in liquid, and solute through membrane. The thick black lines indicate the relative radius of the liquid region (top row) or cell region (bottom row). Plots A and D show the spatiotemporal dependence of temperatures: yellow (light) is high, blue (dark) is low. In subplot A, colors above the thick solid black line indicate temperatures in the solid region. Plots B and E show the spatiotemporal dependence of DMSO concentration, and plots C and F show the spatiotemporal dependence of the NaCl or KCl concentrations, respectively, with blue (dark) indicating low and yellow (light) indicating high concentrations.

Figure 5:
Plots of a numerical simulation of the relative ice/liquid radius (solid) under different solute expansion ratios $\rho = 1 - \rho_s/\rho_l$ and their effects on A at the cell boundary (dashed).

Figure 6:
Plots of a numerical simulation of the relative ice/liquid radius against A evaluated at the cell boundary under different solute expansion ratios $\rho = 1 - \rho_s/\rho_l$.

Figure 7:

Plots of a numerical simulation of the relative osmotically active cell volume under different partition coefficient scenarios. Here the note that equilibrium volumes are only affected when $k_A \neq k_B$, and that because the ice front time-course is short compared to the membrane transport, the dynamics of the $k_A = k_B$ lines overlap (e.g. the $k_A = k_B = 0$ (blue) line is not shown).

Figure 8:

Plots of numerical simulations of the relative radius of the ice–liquid interface (solid lines) and CPA concentration A evaluated at the cell membrane (dashed lines) under different partition coefficient scenarios. Here note that equilibrium volumes are affected by the values of k_A and k_B even for the cases in which $k_A = k_B$ in contrast with the equilibrium cell volumes shown in Figure 7. When $k_A = k_B$ the CPA concentration at the cell membrane increases as the amount of solute rejection decreases (i.e. $k_A = k_B$ increasing from zero); the smallest concentrations at the cell membrane occur when there is complete solute rejection at the solid–liquid interface. When the segregation coefficients differ between the two solutes the result relative to the case of complete solute rejection ($k_A = k_B = 0$) is more complicated.

Figure 9:

Plots of a numerical simulation of the relative ice radius against the concentration at the cell boundary under different partition coefficient scenarios. Here the note that equilibrium volumes are only affected when $k_A \neq k_B$. Note that the final concentrations of A_L are the same when $k_A = k_B$ even though the final ice radius is different when $k_A \neq 0$.

Table 1:

Dimensional parameters for water/ice (H₂O), dimethyl sulphoxide (Me₂SO), sodium chloride (NaCl) and potassium chloride (KCl).

	H ₂ O	Me ₂ SO	NaCl	KCl
L [kJ/kg]	334	55.3–65.7		
k_l [W/(m K)]	0.4	0.2–0.318		
k_s [W/(m K)] (0 °C)	2.22			
(–100 °C)	3.48			
c_{pl} [kJ/(kg K)]	4.2			
c_{ps} [kJ/(kg K)] (0°C)	2.050			
(–100 °C)	1.389			
ρ_L [kg/m ³]	1000			
ρ_S [kg/ m ³] (0 °C)	916.2			
(–100°C)	925.7			
κ^{liq} [m ² /s]	1.4×10^{-7}			
κ^{sol} [m ² /s] (0°C)	1.2×10^{-6}			
(–100 °C)	2.7×10^{-6}			
β [Pa ⁻¹]	5×10^{-10}			
Γ [m]	9.15×10^{-11}			
D [m ² /s]		8×10^{-11}		
k_{diss}		1	1.663	1.772
α_2 [(mol solute/mole total) ⁻¹]		2.423		
α_3 [(mol solute/mole total) ⁻²]		27.231		
β_2 [(mol solute/mole total) ⁻¹]			2.749	0
V_M [m ³ /mol]	1.8×10^{-5}			
m_A [m ³ K/mol]		–0.00186		
m_B [m ³ K/mol]			–0.00309	–0.00330

^a Latent heat for Me₂SO from Choi and Bischof [14].

^b Thermal conductivity values for Me₂SO in Water from Zhang et al [41].

^c Thermal conductivity, heat capacity and density values for ice from www.engineeringtoolbox.com (5-17-2012).

^d Isothermal compressibility β is based on the value for water (inverse bulk modulus of elasticity) from www.engineeringtoolbox.com (9-20-2012).

^e Gibbs-Thomson length scale Γ from Liu *et al.* [28].

^f $T_{MO} = 273.15$ K (0 °C), $T_O = 77.15$ K (–196 °C), $R_C^{init} = 5$ μ m, $R_O = 100$ μ m.

^g Activation energy E_a from 5.56472×10^4 J mol⁻¹ (mouse oocytes) to 3.40996×10^5 J mol⁻¹ (rat hepatocytes) are given by Thirumala and Devireddy [37] for various cell systems.

^h The values of k_{diss} , α_2 , α_3 and β_2 are from Prickett *et al.* [33].

ⁱ The value of DMSO diffusivity D is from Packer *et al.* [32].

Table 2:

Model Parameters and their values.

S	0.8
k_{Is}	0.1 – 0.3
k_{Im}	1
k_{Ic}	1
κ_{Is}	0.08 [0.12 (0°C) – 0.05 (–100°C)]
κ_{Cs}	0.08
k_A	various
k_B	various
Le_A^{liq}	100
Le_B^{liq}	100
Le_A^{cell}	100
Le_C^{cell}	100
$\bar{m}_A$	–0.53
$\bar{m}_B$	–0.88
$\bar{m}_C$	–0.94
ρ	0.08 or 0
$\bar{\rho}$	0.15
Φ_A	0.5
τ	0.2824
ϵ	0.005
$\bar{\Gamma}$	5.4×10^{-8} or 0
$\mathcal{E}$	25 . . . 150
$R_C(t=0)$	5 (μm)
$R_0(t=0)$	100 (μ)
L_p^o	0.41 ($\mu\text{m min}^{-1} \text{atm}^{-1}$)
P_s^o	4.59 ($\mu\text{m min}^{-1}$)
E_a^{Lp}	5.979×10^4 (J/mol)
E_a^{Ps}	6.473×10^4 (J/mol)