Kinetics of Water Loss from Cells at Subzero Centigrade Temperatures

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The cells of which living things are composed and the single-celled microorganisms generally contain very high fractions of water. The high fraction of water in cells introduces a problem when the cells are cooled below 0°C; that is, one has to find out what happens to the water content of the cells when they are cooled to temperatures at which water freezes. There are many factors involved in the process of cooling cells which results in the change of water content in the cells during cooling (9). The scientific work on the cooling of cells has concluded that the biological damage due to cooling was caused by the formation of ice inside the cells. The early workers in this area have suggested that by cooling the cells very rapidly one might produce vitrified water instead of ice inside the cells, and this would prevent biological damage or death. The early workers in this area also showed that some cells and tissues were able to survive at very rapid cooling rates, but they were unable to prove the vitrification process inside the cells. Later, with the discovery of the effect of the cryoprotective agents, the interest in cooling of cells and organs below 0°C for the purpose of banking was increased (10).

When the cells are suspended in an aqueous solution (extracellular fluid) we may distinguish two parts. One part is the extracellular fluid (such as plasma in the case of red blood cells), and the other part consists of the intracellular fluid which is separated from the extracellular fluid by the cell surface membrane. The surface membrane of cells are, generally, very permeable to water, less permeable to neutral solutes of molecular weights up to 200, rather impermeable to cations and almost completely impermeable to large molecules (9). In the case of cooling such a system the formation of ice occurs entirely in the extracellular solution. This is because in the cooling process the extracellular solution is always colder than the intracellular solution, and the nucleation of ice is more probable in the large extracellular space rather than in the small and separated intracellular space. If, for some reason, nucleation occurs inside a cell, ice will be formed in that cell alone, and the other cells will be generally immune to nucleation and, consequently, freezing. Hence, if the cooling of the system is performed at a rather slow rate, one can expect that the nucleation inside the cells (in the intracellular fluid) will be delayed extensively and the intracellular fluids will be in a supercooled liquid state (8, 9). There is another phenomenon involved in the process of cooling, and that is the emigration of water content of the cells due to a driving force. The driving force for water loss from cells could be either the osmotic pres-
sure gradient between the intracellular and extracellular solutions due to "solution effect" or the vapor pressure difference between the supercooled intracellular fluid and the frozen extracellular region. The slower the process of cooling for achieving a certain low temperature, the greater the loss of water from cells to the extracellular space (8, 9). From the above-mentioned observations, one has to choose a certain cooling rate which is slow enough to delay the nucleation process and fast enough to prevent damage to the cells due to extensive water loss.

It is, of course, a well-known fact that the cells are damaged in fast cooling due to nucleation and intracellular freezing (12). But the cells are also damaged in slow cooling due to extensive water loss from the cells (7). The damage to water loss from the cells may be due to the fact that some of the water inside the cells is in the form of hydrates, and as these hydrates decompose they will not be reproduced in the process of thawing. Another cause for the damage to cells is the membrane rupture of the cells in the process of thawing due to cell swelling (4). In red blood cells, and probably in most other kinds of cells, a minimum cell volume is attained after which the cell volume remains constant. Then, in the process of thawing, the now osmotically active intracellular material (due to water loss in the cooling process) will take in the water, swell, and eventually lead to the rupture of the membrane, and, consequently, cell injury.

Mazur (7) has developed a mathematical model for the kinetics of water loss from cells due to vapor pressure differences between the intracellular supercooled fluid and the extracellular frozen space. The basic idea behind Mazur's model is the assumption that the probability of a cell freezing internally is dependent upon the degree of supercooling inside the cell. It is quite likely that the nucleation of ice inside the cells and eventual cell injury due to cooling at subzero centigrade temperatures could be due to other factors besides what is assumed in Mazur's model (1). These other influential parameters may include surface effects related to the proximity of solid substrates, physicochemical properties of the plasma membrane prior to freezing, the presence of intracellular heterogeneous nucleation sites, cell geometry, increased concentrations of intracellular solutes due to the loss of cell water, and the presence of a cryophylactic agent (1).

To facilitate the formulation of the problem, Mazur (7) assumes that the protoplasm behaves like an ideal dilute solution, i.e., Raoult's law is applicable, the intracellular space has uniform temperatures during the cooling process, the plasma membrane is intact and there is no shrinkage of the cell surface due to the loss of water from the cell, the plasma membrane is permeable only to water, and the cooling rate is constant during the cooling process. While some of the above assumptions are expected to be close to reality, a number of them need reevaluation (1). The protoplasm is not generally an ideal solution, and the temperature inside the cells is not uniform during the cooling process, and actually there is a temperature gradient inside the cells in the radial direction. Also, due to water loss the surface area of the cells shrink and actually up to a certain water-fraction loss (which depends on the kind of cells) from the cells, the amount of shrinkage of the surface area of the cells will be proportional to the volume of the lost water from the cells. The other assumptions made by Mazur (7) are quite satisfactory, and practically they could be achieved by the proper choice of medium for extracellular substance and the mechanical control of the cooling rate.

In the present report a model is proposed for which it is assumed that the protoplasm is a nonideal solution, the surface
cell-membranes shrink upon water loss from the cells, and that there is a temperature gradient in the radial direction of the intracellular space during the cooling process. There is also another refinement made on this model, and that is the application of the exact vapor pressure equations for ice and water instead of the approximated relations used by Mazur based on the Clausius–Clapeyron equation (7).

**THEORY**

In the mathematical model which is proposed in the present report, it is assumed that the cells are spherical in shape with their surface membranes permeable to water only. It is also assumed that the protoplasm consists of a nonideal solution and that the extracellular space is in the frozen state. Consequently, the transfer of water from inside to outside the cells will be due to the difference between the vapor pressure of ice in the extracellular space and the partial pressure of water in the intracellular (protoplasm) solution. Since the protoplasm is assumed to be a non-ideal solution, the following relation will hold for the partial pressure of water in the intracellular space (11).

$$p_i = \gamma w x_w p_w$$  \hfill (1)

In the above equation, $\gamma w$, $x_w$, and $p_w$ are the activity coefficient, the mole fraction, and the vapor pressure of water in the protoplasm, respectively. We may consider the protoplasm as a binary solution (water and a nonwater component). This is due to the assumption made that during the cooling process no substance other than water will leave the protoplasm. We can then approximate the activity coefficient of water by the following equation (11).

$$\ln \gamma w = \Delta/RT (1 - x_w)^2.$$  \hfill (2)

The above equation is called the two-suffix Margules equation. The coefficient $\Delta$ is an empirical constant, positive or negative, with units of energy, characteristic of protoplasm, which depends generally on temperature, but not composition. Due to the lack of experimental data and as an approximation, one may consider $\Delta$ as a constant.

By taking the logarithm of both sides of Eq. (1) and replacing Eq. (2) for $\gamma_w$, we get:

$$\ln p_i = \ln (x_w p_w) + \left(\frac{\Delta}{RT}\right) (1 - x_w)^2.$$  \hfill (3)

The vapor pressure of water, $p_w$, as a function of temperature for the temperature range of $-5^\circ\mathrm{C}$ to the critical point of water is given by an empirical equation which is proposed by Osborne and Meyer (2, p. 574), and it is given in the Appendix by Eq. (A-1). For temperatures below $-5^\circ\mathrm{C}$, we may extrapolate this equation, and, due to the lack of experimental data on this range, this will be the best choice possible for the vapor pressure of supercooled water (3). Also, the vapor pressure of ice, $p_{ice}$, as a function of temperature is given by an empirical equation proposed by Washburn (2, p. 598), and this is also given in the Appendix by Equation (A-2). The extracellular medium consists of ice and the partial pressure of $\mathrm{H}_2\mathrm{O}$ in the extracellular medium, $p_e$, will be equal to $p_{ice}$. With the use of Eqs. (3), (A-1), and (A-2), we can derive the following equation for the ratio of the partial pressures of $\mathrm{H}_2\mathrm{O}$ in the extracellular and intracellular spaces:

$$\ln \left(\frac{p_e}{p_i}\right) = -\ln x_w - \left(\frac{\Delta}{RT}\right) (1 - x_w)^2 + \ln \left(\frac{p_{ice}}{p_w}\right)$$  \hfill (4)

If we consider $V_w$ as the total volume of water in the cell, we will have

$$V_w = n_w \bar{\nu}_w$$

where $\bar{\nu}_w$ is the partial molar volume of water in the protoplasm

$$\bar{\nu}_w = \left(\frac{\partial V_p}{\partial n_w}\right)_{T, p, n_{prot}}$$

$n_w$ is the total number of moles of water in the protoplasm, $V_p$ is the total volume of protoplasm, and $n_{prot}$ is the total number of
moles of non-water part of the protoplasm. According to the thermodynamics of multi-component systems we can write

\[
\left( \frac{\partial \ln \gamma_w}{\partial P} \right)_{T,x_w} = \frac{\bar{v}_w - v_w}{RT} = \frac{v_w^K}{RT}
\]

With the consideration of Eq. (2) for \( \gamma_w \), we get

\[
\left( \frac{\partial \ln \gamma_w}{\partial P} \right)_{T,x_w} = 0
\]
or,

\[
\frac{\delta}{\delta P} T = x_w
\]

where \( v_w \) is the specific volume of pure water at the same temperature and pressure as the protoplasm. Consequently, for the mole fraction of water in the protoplasm we can write

\[
x_w = \frac{(n_{w0}v_w0)/(n_{w0} + n_{z0}v_{z0})}{(V_w0)/(V_w0 + n_{z0}v_{z0})}.
\]

The above relation for \( x_w \) is similar to the equation derived by Mazur (7) in the case when it was assumed that the protoplasm behaved like an ideal solution. By replacing Eq. (5) into Eq. (4) we get

\[
\ln \left( \frac{P_e}{P_i} \right) = -\ln \left( \frac{V_w}{V_w0 + n_{z0}v_{z0}} \right) - \frac{\Delta}{RT} \left( \frac{n_{z0}v_{z0}}{V_w0 + n_{z0}v_{z0}} \right)^2 + \ln \left( \frac{P_{ice}}{P_w} \right).
\]

The rate at which the volume of the intracellular water changes can be represented by the following equation.

\[
\frac{dV_w}{dt} = \frac{kRT}{v_w} \ln \left( \frac{P_e}{P_i} \right).
\]

where \( k \) is the permeability constant of the cell-surface membrane, and \( A \) is the surface area of the membrane. The permeability constant, \( k \), changes with the change of temperature and generally it can be shown by the following relation.

\[
\ln \left( \frac{k}{k_0} \right) = b(T - T_0) - c\left( \frac{1}{T} - \frac{1}{T_0} \right)
\]

In the above equation, \( k_0 \) is the permeability constant at the reference temperature, \( T_0 \), and \( b \) and \( c \) are constants which can be found experimentally. The surface area, \( A \), of the cell membrane changes as the water content of the cell changes.

If we denote the cell surface area and the cell volume at the beginning of cooling process as \( A_0 \) and \( V_{p0} \), and at time \( t \) as \( A \) and \( V_p \), respectively, we can write the following relation between the surface and volume of the cell

\[
\frac{A}{A_0} = \left( \frac{V_p/V_{p0}}{2} \right)^{2/3}
\]

This relation is true only when the geometric shape of the cell is retained after the volume change. Now if we denote the total volume of the water content of the cell at the beginning of cooling as \( V_{w0} \) and at time \( t \) as \( V_w \), we can write the above equation in the following form

\[
A = A_0 \left[ (V_w/V_{w0} + \alpha)/(1 + \alpha) \right]^{2/3}
\]

where \( \alpha = V_{p0}/V_{w0} - 1 \).

By joining Eqs. (6), (7), (8), and (9) we get the following relation for the rate of change of the volume of the intracellular water

\[
\frac{dV_w}{dt} = \frac{k_0A_0RT}{v_w} \exp \left\{ b(T - T_0) \right\} \left[ \frac{V_w/V_{w0} + \alpha}{1 + \alpha} \right]^{2/3} \times \ln \left( \frac{P_e}{P_i} \right).
\]

**Rate of Cooling**

The assumption made in this work is that the rate of cooling on the surface of the cell is a constant, that is:

\[
\frac{dT}{dt} = B \text{(a constant)}; \quad \text{on the cell surface.}
\]

This assumption is different from the one which is made by Mazur (7, 8). In the case of Mazur's model it is assumed that the rate of cooling of the cell, as a whole, is a constant. That assumption confines the cell temperature to be uniform all over the
cell during the cooling process and implies that the thermal conductivity of the cell is infinity which of course is unrealistic. The assumption presented by Eq. (11) does not restrict the cell to be in uniform temperature states during the cooling process and it is realistic. In order to find the temperature profile inside the cell and the average temperature of the cell (as a function of surface cell temperature and the rate of cooling of the cell surface), we must use the principle of heat transfer. Based on the Fourier's law of heat conduction, the following equation has been derived (6) for the average temperature of a sphere where its surface cooling (or heating) is performed at a constant rate, $B$, and where its temperature at the beginning of the cooling (or heating) process (at $t = 0$) is equal to $T_0$:

$$
\frac{T - T_0}{T_0} = Pd \left\{ F_0 - \frac{1}{15} \right. \\
+ \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^4} \exp \left\{ - n^2 \pi^2 F_0 \right\}. 
$$

(12)

In the above equation, $Pd$ stands for the Predvoditelev number and $F_0$ stands for the Fourier number as defined by the following relations

$$
Pd = \frac{(BD^2/4aT_0)}{(BA/4\pi a T_0)}, \quad (13)
$$

and

$$
F_0 = \frac{(4at/D^2)}{4\pi a/\Delta}. \quad (14)
$$

In the above relations, $D$ stands for the cell diameter, $t$ stands for the cooling (or heating) time, and $a = k/\rho C_p$ stands for the thermal diffusivity of the protoplasm; $k$, $\rho$, and $C_p$ are thermal conductivity, density, and heat capacity of the protoplasm, respectively.

Differential Eq. (10), together with auxiliary Eqs. (6) and (12) can be solved in order to get the relation between the volume of the water content of the cell and the cell temperature during the cooling process. For this purpose the numerical values of parameters $T_0$, $B$, $\nu_w$, $b$, $c$, $\alpha$, $V_{w0}$, $k_0$, $a$, $\Delta$, $n_2$, and $A_0$ are needed. $T_0$ is the temperature of the system at the start of the cooling process, and for normal (atmospheric) pressure it is equal to 273.16°K (or 0°C). Parameter $B$, the cooling rate of the cell surface, is at our disposal. For the remaining ten parameters there are little or no experimental data available (1, 7). For this reason, it is quite helpful to introduce the above equations in dimensionless forms. This will enable us to formulate the rate Eq. (10) and the auxiliary Eqs. (6) and (12) with respect to a number of dimensionless parameters fewer than the 12 parameters presented above.

It should be mentioned that in the formulation which is presented above the only part for which it is assumed that the cell is spherical is for the derivation of Eq. (12). For nonspherical cells it is generally possible to derive a relation, similar to Eq. (12), for the average temperature of the cell as a function of the cooling rate of the cell surface and the cooling time.

**Dimensionless Relations**

In order to simplify the calculations, we introduce the above equations in dimensionless forms. For this purpose we define the dimensionless variables $V^*$, $T^*$, and $t^*$ by the following relations

$$
V^* = \frac{V_w}{V_{w0}},
$$

(15)

$$
T^* = \frac{T - T_0}{T_0},
$$

(16)

and

$$
t^* = -\frac{Bt}{T_0}.
$$

(17)

We also define the following dimensionless parameters

$$
B^* = -\frac{(\nu_w BV_{w0})}{(k_0 A_0 R T_0^2)},
$$

(18)

$$
b^* = b T_0,
$$

(19)

$$
c^* = c/T_0,
$$

(20)

$$
n_2^* = n_2 \nu_w/V_{w0},
$$

(21)

$$
\Delta^* = \Delta/RT_0,
$$

(22)

$$
Pd^* = (BA_0)/(4\pi a T_0).
$$

(23)
Consideration of the above dimensionless variables and parameters, Eqs. (6), (10), and (12) will be, respectively, as follows:

\[
\ln \left( \frac{p_e}{p_i} \right) = - \ln \left( \frac{V^*}{V^* + n_2^*} \right) \left( \frac{\Delta^*}{T^* + 1} \right) \left( \frac{n_2^*}{V^* + n_2^*} \right) + \ln \left( \frac{P_{ic1}}{P_{w1}} \right), \tag{24}
\]

\[
\frac{dV^*}{dt^*} = - \left( \frac{T^* + 1}{B^*} \right) \exp \left( b^* T^* \right) \left( \frac{V^* + \alpha}{1 + \alpha} \right)^4 \ln \left( \frac{p_e}{p_i} \right) \tag{25}
\]

and

\[
T^* = - t^* + Pd^0 \left( \frac{V^* + \alpha}{1 + \alpha} \right)^\frac{4}{3} \left\{ - \frac{1}{15} + \frac{6}{\pi^4} \sum_{n=1}^{\infty} \frac{1}{n^4} \exp \left( -n^2 \pi^2 F_0 \right) \right\} \tag{26}
\]

The Fourier number, \( F_0 \), can be represented with respect to the defined dimensionless groups by the following relation

\[
F_0 = \frac{t^*}{Pd^0 \left( \frac{1 + \alpha}{V^* + \alpha} \right)^\frac{4}{3}} \tag{27}
\]

It should also be mentioned that \( n_2^* \) and \( \alpha \) are related to each other by the following relation: \( n_2^* = (v_w/v_2 M_2) \alpha \), where \( v_w \) and \( M_2 \) are specific volume and molecular weight of the nonwater part of the protoplasm, respectively. Due to the lack of rigorous data on \( v_w \) and \( M_2 \) we take \( n_2^* \) as an independent parameter in the present report.

With the knowledge of the numerical values of the dimensionless parameters \( B^* \), \( b^* \), \( c^* \), \( n_2^* \), \( \alpha \), \( \Delta^* \), and \( Pd^0 \), it will be possible to solve Eq. (25) together with the auxiliary relations, Eqs. (24) and (26), in order to derive the relation between \( V^* \) and \( T^* \). As it is shown above, we have been able to reduce the number of parameters from 12 to seven by introducing them in dimensionless groups. The numerical values of the dimensionless parameters (18–23) are dependent on the nature of the cells under consideration. The ranges of variation of the numerical values of the dimensionless variables and parameters for different cells are reported on Table 1. The ranges of variation of \( B^* \), \( b^* \), \( c^* \), \( n_2^* \), \( t^* \), \( T^* \), \( V^* \), and \( \alpha \) are according to the data compiled by Mazur and reported in Refs. (7) and (8). It is somewhat difficult to choose the values of \( Pd^0 = B A/4 \pi a T_0 \) which could be realistic for cells. We know that the maximum value of \( Pd^0 \) is zero (when \( a = \infty \) and/or \( B = 0 \)). The minimum value of \( Pd^0 \) can be found by replacing the minimum value of \( B \) in the relation for \( Pd^0 \) \( (B_{min} = 10^{-4} \, 0^\circ \text{C/min} \) according to Mazur (7)). The value of \( a \) for liquids can vary from \( 10^{-2} \) to \( 10^{-4} \, \text{ft}^2/\text{hr} \) depending on the kind

### Table 1

**Ranges of Variation of the Dimensionless Groups (Parameters and Variables) Used in the Calculations Presented in the Text**

<table>
<thead>
<tr>
<th>Dimensionless group</th>
<th>Range of variation</th>
<th>Values chosen</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B^* )</td>
<td>0–50</td>
<td>5 \times 10^{-3}, 5 \times 10^{-3}, 0.5, 5, 50</td>
</tr>
<tr>
<td>( b^* )</td>
<td>0–35</td>
<td>0, 5, 15, 25, 35</td>
</tr>
<tr>
<td>( c^* )</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( n_2^* )</td>
<td>0–0.2</td>
<td>0, 0.002, 0.01, 0.02</td>
</tr>
<tr>
<td>( Pd^0 )</td>
<td>0–10^{-2}</td>
<td>0, -0.001, -0.01, -0.1, -1, -10</td>
</tr>
<tr>
<td>( t^* )</td>
<td>0–0( \infty )</td>
<td>0–( \infty )</td>
</tr>
<tr>
<td>( T^* )</td>
<td>0–0.10</td>
<td>0, -0.01, -0.02, ..., -0.10</td>
</tr>
<tr>
<td>( V^* )</td>
<td>0–1</td>
<td>0, 0.1, 0.2, ..., 1.0</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>0–1</td>
<td>0, 0.2, 0.8</td>
</tr>
<tr>
<td>( \Delta^* )</td>
<td>-1.5–+1.5</td>
<td>-1.5, -0.5, 0, 0.5, 1.5</td>
</tr>
</tbody>
</table>
of liquid. If we choose $A = 1964 \ \mu m^2$ (the largest value according to Mazur (7)), and $T_0 = 273^\circ K$, then the minimum value of $Pd^*$ will be of the order of $-10^{-2}$. The range of variation chosen for $\Delta^*$ is arbitrary due to the lack of experimental data on the activity coefficient of water in protoplasm.

RESULTS AND DISCUSSION

In the case when the protoplasm is considered as an ideal solution ($\Delta^* = 0$), the cells are considered with uniform temperature during the cooling process ($Pd = 0$, $Fo = \infty$ or infinite thermal conductivity), and the cell surface is considered intact and not a function of water content of the protoplasm ($A = A_0$), Eqs. (24) to (27) will be joined and simplified to the following relation

$$\frac{dV^*}{dT^*} = -\left(\frac{T^* + 1}{B^*}\right) \exp\left\{b^*T^* + \frac{c^*T^*}{1 + T^*}\right\} \times \left\{-\ln\left(\frac{V^*}{V^* + n_2^*}\right) + \ln\left(P_{\text{ice}}/P_w\right)\right\}.$$  

(28)

This equation is equivalent to the relation based on Mazur's model in dimensionless form for $c^* = 0$ and with the consideration of Clausius–Clapeyron equation for the vapor pressures of ice and water (7, 8).

Figure 1 is plotted by the numerical solution of differential Eq. (28) for the percentage of the intracellular water remaining inside the cell at various temperatures and cooled at several rates. The reason for not introducing the parameters and variables in dimensionless forms in Fig. 1 is to compare the results with Mazur's results. The data for the parameters used in plotting this figure are equivalent to the data used by Mazur in plotting Fig. 2 of Ref. (7). In Fig. 1 the deviations of the results of Mazur's calculations (Fig. 2 or Ref. (7)) from the results of Eq. (28) are shown by dashed lines. Figure 1 indicates that the difference between the effects of the Clausius–Clapeyron equation (as used by Mazur) and the exact relations for vapor pressures of ice and water (as used in the present report) on the prediction of cell water content may be significant at high cooling rates only.

As it is mentioned above in Mazur's model, Eq. (28), it is assumed that the cell membrane surface area remains constant as water is emigrated out from the cell, the cells have uniform temperatures during cooling process, and that the protoplasm is an ideal solution. In order to observe the effect of each of these assumptions on the prediction of the variation of the volume of the water content of the cell versus temperature, we consider the corrections on...
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Fig. 2. Calculated fractions of supercooled intracellular water remaining in the cells at various temperatures cooled with the assumption that the protoplasm is an ideal solution \( \Delta^* = 0 \), and that the inner-cell temperatures remain uniform spatially during the cooling process \( (P_d^* = 0) \), according to different models, with \( B^* = 0.5 \), \( n_2^* = 0.01 \) and \( b^* = c^* = 0 \). (1) Mazur’s model, Eq. (28); (2) The present model with \( \alpha = 0.2 \), Eq. (29); (3) The present model with \( \alpha = 0.8 \), Eq. (29); (4) The present model assuming that the protoplasm consists of pure water, \( n_2^* = \alpha = 0 \), Eq. (29).

Each of these assumptions separately and we solve the rate equation accordingly.

Effect of the Membrane Surface Area Change

If we assume that the cells have uniform temperatures during the cooling process and that the protoplasm is an ideal solution, Eqs. (24)–(26) will reduce to the following equation

\[
\frac{dV^*}{dT^*} = -\left(\frac{T^* + 1}{B^*}\right) \exp\left\{b^* T^* + \frac{c^* T^*}{1 + T^*} \left(\frac{V^* + \alpha}{1 + \alpha}\right)^{\frac{3}{2}}\right\} \times \left\{ -\ln\left(\frac{V^*}{V^* + n_2^*}\right) + \ln \left(\frac{P_{ice}}{P_w}\right) \right\}.
\]

(29)

The difference between this equation and Mazur’s model, Eq. (28), is that in the above equation the effect of the membrane surface change due to water loss is considered while in Mazur’s model it is assumed that the membrane surface area remains constant. In order to demonstrate the effect of surface membrane change on the rate of water loss Fig. 2 is plotted. As it is clear from Fig. 2 consideration of the surface membrane change due to water loss in the equation for the rate of water loss is necessary and the effect of this correction on the prediction of the rate of water loss is appreciable. The dashed-line on Fig. 2 is for the case when it is assumed that the protoplasm is 100% pure water \( (\alpha = 0; n_2^* = 0) \). Value of \( \alpha = V_{p^*}/V_{w^*} - 1 \) is an indication of the water content of the cell at the beginning of the cooling process.

Effect of Nonideality of Protoplasm

If the only assumption made is that the cell temperature remains uniform inside the cell during the cooling process, Eqs. (24)–(26) will be joined together and they will reduce to the following relation

\[
\frac{dV^*}{dT^*} = -\left(\frac{T^* + 1}{B^*}\right) \exp\left\{b^* T^* + \frac{c^* T^*}{1 + T^*} \left(\frac{V^* + \alpha}{1 + \alpha}\right)^{\frac{3}{2}}\right\} \times \left\{ -\ln\left(\frac{V^*}{V^* + n_2^*}\right) - \left(\frac{\Delta^*}{T^* + 1}\right) \times \left(\frac{n_2^*}{V^* + n_2^*}\right)^2 + \ln \left(\frac{P_{ice}}{P_w}\right) \right\}.
\]

(30)

The difference between this equation and Eq. (29) is that in this equation the effect of the nonideality of the protoplasm on the water content of the cell is also taken into account. In Eq. (30), \( \Delta^* \) is a measure of nonideality of protoplasm and, for \( \Delta^* = 0 \), we get the same results as for Eq. (29) in which it is assumed that the protoplasm is an ideal solution. In order to study the effect of nonideality of protoplasm on the variation of cell water content vs temperature, Fig. 3 is plotted for different positive and negative values of \( \Delta^* \). Again, in this figure in order to uncomplicate the com-
Fig. 3. Effect of the nonideality of protoplasm on the amount of water remaining in the cells at various temperatures with the assumption that the inner-cell temperatures remain uniform spatially during the cooling process \((Pd^* = 0)\), with \(B^* = 0.5, n_\alpha^* = 0.01, \) and \(b^* = c^* = 0\). The solid lines are for \(\alpha = 0.8\) and the dashed lines are for \(\alpha = 0.2\).

Fig. 4. Effect of the nonuniformity of temperature inside the cells on the amount of water remaining in the cells at various temperatures with the assumption that the protoplasm is an ideal solution \((\Delta^* = 0)\), with \(B^* = 0.5, n_\alpha^* = 0.01, b^* = c^* = 0; \) and \(\alpha = 0.2\).

Fig. 5. The same as for Fig. 4, except that in this figure, \(\alpha = 0.8\).

Effect of Nonuniformity of Temperature Inside the Cells

When it was assumed that the cell was at a uniform temperature during the cooling process, it was implied that the thermal diffusivity, \(a\), (or thermal conductivity, \(k\)) of the cell protoplasm was infinity, and consequently, \(Pd^* = 0\). It is a clear fact that the thermal diffusivity of the cell proto-

Fig. 6. Calculated fractions of supercooled intracellular water remaining in the cells at various temperatures with cell-surfaces cooled at different rates as labeled. For this figure \(n_\alpha^* = 0.01, b^* = 5, c^* = 0, \alpha = 0.2, \) and \(Pd^* = 0\). The solid lines are for \(\Delta^* = 0.5, \) and the dashed lines are for \(\Delta^* = -0.5\).
plasm can not be infinity, and that it has a limited value. Based on the expected range of variation of thermal diffusivity of cell protoplasts, it is shown in an earlier part of this report that the expected range of variation of $Pd^\circ$ for cell protoplasts is $0-10^{-2}$. Figures 4 and 5 are plotted in order to study about the effect of the consideration of nonuniformity of temperature inside the cells on the mathematical modeling of the kinetics of water loss from cells during the cooling process. These figures are results of the solutions of Eqs. (24)-(26) for different values of $Pd^\circ$ as shown on the figures. Figure 4 is for $\alpha = 0.2$ (cells with 83.3% water in their protoplasm at the beginning of cooling) and Fig. 5 is for $\alpha = 0.8$ (cells with 55.6% water in their protoplasm at the beginning of cooling). In both of these figures it is assumed that the cell membrane permeability constant is independent of temperature ($b^* = c^* = 0$) and that the protoplasm is an ideal solution ($\Delta^* = 0$) in order to uncomplicate the comparisons of different curves with different $Pd^\circ$’s. Figure 4 indicates that the cooling curves for $Pd^\circ = 0, -0.001, -0.01$ are the same, while Fig. 5 indicates that the cooling curves for $Pd^\circ = 0, -0.001$ are the same. With the consideration of the fact that the realistic value of $Pd^\circ$ for protoplasm is in the range of $0-10^{-2}$, we may conclude from Figs. 4 and 5 that the consideration of the effect of nonuniformity of temperature inside the cells in the mathematical modeling of the kinetics of water loss from cells during cooling process may be important only for the cells which have low fractions of water in their protoplasm before the start of the cooling process. It should be mentioned that actually most of the cells of which the living things are composed of, and also, most of the single-celled microorganisms contain high fractions of water in their protoplasm.

**The General Case**

In order to study the variation of water content of the cell vs temperature, in the general case when the membrane surface area changes, the protoplasm is considered a nonideal solution and the internal cell temperature is nonuniform during the cooling process, Figs. 6–8 are plotted. These figures are based on the solutions of Eqs. (24)-(26) for $n^\circ = 0.01$, $b^* = 0.5$, $c^* = 0$, $\Delta^* = +0.5$, $\alpha = 0.2$, and $B^* = 0.05, 0.5, 5, 50$. The values chosen for these parameters are in the ranges of the values reported in Table 1, and the choices made (except for $\Delta^*$ which is arbitrary) are based on the experimental data reported by
Mazur (7, 8). Figure 6 is for $Pd^0 = 0$, $-0.001$, and $-0.01$ and it indicates that for cells with 83.3% water content at the beginning of cooling (which is close to the real value of water content for most cells) the effect of the consideration of the non-uniformity of temperature inside the cells in the mathematical modeling of the kinetics of water loss from cells at subzero degree centigrade, for the prediction of rate of water loss, is negligible. Consequently, for these kinds of cells (cells with high fractions of water) we can effectively neglect the terms due to the nonuniformity of temperature inside the cells and work with Eq. (30) instead of Eqs. (24)-(26). This will also greatly reduce the computations necessary for the prediction of the water loss from cells at subzero centigrade temperatures.

Figure 7 is for $Pd^0 = -0.1$, and Fig. 8 is for $Pd^0 = -1.0$. Of course any value of $Pd^0$ less than $-10^{-2}$ seems to be out of the expected range of $Pd^0$ for living cells, but Figs. 7 and 8 are plotted in order to have a qualitative picture about the trend of the changes in the cooling curves with respect to the changes of $Pd^0$. It should be mentioned, again, that there is no criteria by which one could estimate the values of $\Delta^*$ for different cell protoplasms. This is due to the lack of knowledge about the nature of the nonideality of the protoplasm solutions. The values chosen for $\Delta^*$ are arbitrary, but it may very well happen that the real value of $\Delta^*$ for different cells be different from the chosen range. Further experimental research is necessary in order to establish the role of water inside the cells and the measurement of the nonidealities of the protoplasm solutions.

**SUMMARY**

A mathematical model is developed for the calculation of the kinetics of water loss from cells at subzero centigrade temperatures. In this model it is assumed that the cell surface membrane is permeable to water only, the protoplasm is a nonideal solution, the cells are spherical, and during the cooling process the cell temperature is not uniform inside the cell. It is also assumed that because of water loss due to cooling process the cell volume and the cell surface area reduce and the reductions in surface area and volume of the cell are functions of the amount of water loss from the cell. Based on this model, and for different conditions, the fractions of supercooled intracellular water remaining in the cells at various temperatures are calculated.

It is shown that for cooling cells at subzero centigrade temperatures (1) the consideration of Clausius–Clapeyron equation for vapor pressures of water and ice, instead of the exact vapor pressure relations, may produce errors in the prediction of the amount of water loss from the cells at high cooling rates only, (2) the assumption of intact cells will produce considerable deviation in the prediction of water loss from the cells as compared to the more realistic assumption of shrinkable cells, (3) the non-ideality of protoplasm solution is very effective on the prediction of the amount of water loss from the cells, and (4) the assumption of uniform-temperature cells during the cooling process may be erroneous only for cells with small fractions of water in their protoplasms.

**APPENDIX**

According to Oshbome and Meyers (Dorsey, p. 574, Ref. (9)), the vapor pressure of water can be described by the following relation

$$
\ln P_w = 2.3026\left[A_1 + \frac{B_1}{T} + \frac{C_1T}{T(10^{D_1T} - 1)} + E_1 \cdot 10^{F_1T^{1/4}}\right]
+ \ln(760), \quad (A-1)
$$
where \( P_e \) is in mm Hg and \( T \) is in °K, and,
\[
\begin{align*}
x &= T^2 - K, \\
K &= 293700, \\
y &= 647.27 - T, \\
A_1 &= 5.4266514, \\
B_1 &= -2005.1, \\
C_1 &= 1.3869 \times 10^{-4}, \\
D_1 &= 1.1965 \times 10^{-11}, \\
E_1 &= -0.0044, \\
F_1 &= -0.0057148.
\end{align*}
\]

The above equation holds for -5°C until the critical point of water. For temperatures under -5°C, the above equation can be extrapolated.

The vapor pressure of ice is presented by the following relation which is derived by Washburn (2, p. 598, Ref. 9)
\[
\ln P_{\text{ice}} = 2.3026\left[\left(\frac{A_2}{Z}\right) + B_2 \ln Z + C_2Z + D_2Z^2 + F_2\right], \quad (A-2)
\]

where \( P_{\text{ice}} \) is in mm Hg, and
\[
\begin{align*}
Z &= T - 0.06, \\
A_2 &= -2445.5646, \\
B_2 &= 8.2312/2.3026, \\
C_2 &= -1677.006 \times 10^{-5}, \\
D_2 &= 120514 \times 10^{-10}, \\
E_2 &= -6.757169,
\end{align*}
\]
and where \( T \) is in °K.

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