A GRAPHICAL METHOD FOR DETERMINING THE OPTIMAL CRYOPRESERVATION RATE OF AN ARBITRARY BIOLOGICAL SYSTEM

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ABSTRACT

The effect of various parameters on the predicted optimal cooling rate (B_{opt}) of an arbitrary biological system has been studied using a well-defined water transport model. The various parameters investigated are apparent activation energy (E_{Lp}), reference permeability of the membrane to water (L_{pg}), osmotically inactive cell volume (V_b), and the diameter of a spherical cell (D). B_{opt} is determined assuming a damaging criterion for initial percentage of water trapped ($\%W_T$), along with the end temperature at which water transport is assumed to cease (T_{end}). For each parameter a physiologically relevant range is selected (see Table. 1).

A significant observation of this study is that there is an exact inverse relationship between the ratio of initial volume of intracellular fluid (water) to the surface area available for water transport (denoted as, WV/SA) and the predicted optimal cooling rate (B_{opt}). This relation is then used to develop a Generic Optimal Cooling Rate Chart (GOCRC) with the activation energy (E_{Lp}) as the abscissa and a cooling rate (B_{graph}) as the ordinate. By using this GOCRC we can calculate B_{opt} values for any combination of other given parameters assuming a predetermined value for $\%W_T$ and T_{end} (in our specific case these being 5% and –45 ˚C respectively), by using a simple mathematical equation.

Table 1: Physiologically Relevant Range of Model Parameters

Parameter	Range Selected	Step Size
L_{pg}	0.01 to 100.0 µm/min-atm	10.0 µm/min-atm
E_{Lp}	4.0 to 100.0 Kcal/mole	2.0 Kcal/mole
V_b	$0.2V_o$ to $0.8V_o$	$0.2V_o$
T_{end}	-45 ˚C to –15 ˚C	-15 ˚C
D	5.0 to 50.0 µm	5.0 µm

INTRODUCTION

The rate of cooling is an important determinant of cell survival in a cryopreservation process. It is observed that both slow freezing and rapid freezing are lethal to cell survival and maximum % of survival is achieved at an intermediate rate. This intermediate cooling rate is referred as the 'Optimal Cooling Rate'. Mazur [3] formulated a two-factor hypothesis to explain the two mechanisms that are responsible for cell injury/survival during slow and rapid cooling processes; a) at slow cooling rate, injury occurs due to cellular dehydration, i.e., due to water transport from the cell, (b) at high cooling rate, cellular damage is associated with the formation of ice inside the cells, i.e., by Intracellular Ice Formation (IIF). Several mathematical models have been developed to account for both dehydration and intracellular ice formation in a biological system [1-5]. A well-accepted model for cellular dehydration is given below.

Mathematical model of water transport:

Mazur [2] developed a mathematical model for the volumetric change in cells due to the water transport during freezing process in the presence of extracellular ice. The reduction in the cellular volume, due to the loss of intracellular water is modeled thermodynamically as,

$$\frac{dV}{dT} = -\frac{L_p A_c RT}{B\nu_w}\left[\ln\frac{(V-V_b)}{(V-V_b)+\varphi_s n_s \nu_w} - \frac{\Delta H_f}{R}\left(\frac{1}{T_r}-\frac{1}{T}\right)\right]$$

where L_p is the plasma cell membrane permeability to water and is defined as [1],

$$L_p = L_{pg}\exp\left(-\frac{E_{Lp}}{R}\left(\frac{1}{T}-\frac{1}{T_R}\right)\right)$$

A detailed description and the various assumptions made in the development of the water transport model are discussed elsewhere [1-5] and are beyond the scope of this abstract.

RESULTS AND DISCUSSION

In this study we found an exact linear relationship between the change in L_{pg} and corresponding change in B_{opt}. The relationship between B_{opt} and the other investigated parameters namely E_{Lp}, V_b, T_{end}, $\%W_T$ and D is shown in Table. 2. Interestingly, at higher values of E_{Lp} ($\geq$50 kcal/mole) the variation in B_{opt} is very small ($\pm$5%) irrespective of the change in other parameters.

Table 2: Effect of Various Model Parameters on B_{opt}

Parameter	Change in value	Change in 'B_{opt}'	Nature of relation
L_{pg}	+ve	+ve	Linear
E_{Lp}	+ve	-ve	Nonlinear
V_b	+ve	+ve	Nonlinear
T_{end}	+ve	-ve	Nonlinear
D	+ve	-ve	Nonlinear
$\%W_T$	+ve	+ve	Nonlinear

Further analysis of the simulated optimal cooling rate data revealed that the predicted B_{opt} values are constant for a given ratio of the initial volume of intracellular fluid to the surface area available for water transport (WV/SA) during the freezing process (as shown in Fig.1). At a given E_{Lp} value the B_{opt} value has a linear inverse variation with the WV/SA ratio. For example the B_{opt} value at WV/SA=1.0 and E_{Lp}=30 Kcal/mole is 228 ˚C/min and when the value WV/SA reduces by a factor 5 the B_{opt} increases by the same factor of 5 (i.e., at WV/SA =0.2, the B_{opt} is 1140 ˚C/min). This inverse relation is found to extend to any ratio of water volume to surface area (WV/SA).

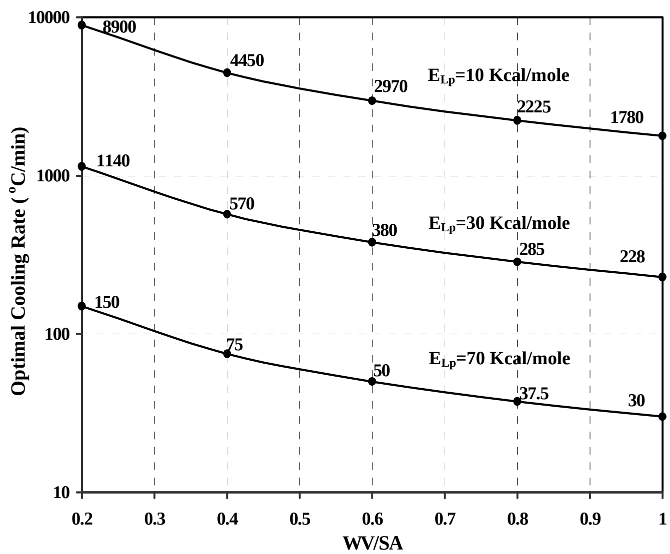

Figure 1: The variation of B_{opt} with WV/SA at different E_{Lp} values. The data is shown at L_{pg}=1.0 μm/min-atm, T_{end}= -45 ˚C and $\%W_T$ = 5.0

The observed relationship between WV/SA and B_{opt} can be used to collapse all of the curves shown in Fig.1 into a single curve as shown in Fig.2. We call Fig.2 as the Generic Optimal Cooling Rate Chart (GOCRC). The optimal cooling rate (B_{opt}) for any biological system can be calculated using a simple mathematical relation shown inside of the Fig.2. The 'B_{opt}' in the LHS of the equation is the optimal cooling rate to be calculated and 'B_{graph}' the first term in the RHS of the equation is the cooling rate shown on the Y-axis of the GOCRC. Significantly, the GOCRC can be used irrespective of the cell geometry (spherical, cylindrical or a Krogh cylinder) as long as the physiologically relevant data is provided.

As stated earlier, to generate the GOCRC (Fig. 2), we assigned values to two model parameters: T_{end} (-45˚C) and $\%W_T$ (5%). However, similar GOCRC's for other values of T_{end} and $\%W_T$ have been generated for appropriate use (data not shown). The major limitation of the GOCRC's is the non-inclusion of IIF (intracellular ice formation) in our model to predict the B_{opt} values.

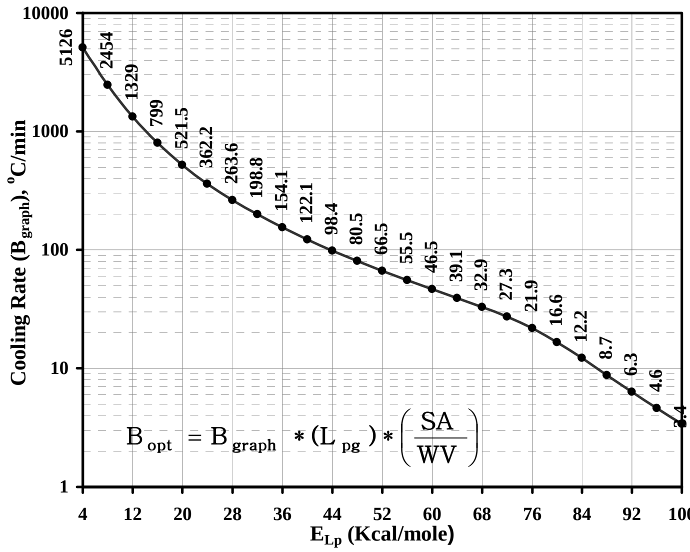

Figure 2: Generic Optimal Cooling Rate Chart (GOCRC).

CONCLUSION:

The effect of various parameters on the predicted optimal cooling rate of an arbitrary biological system has been thoroughly investigated using a well-defined water transport model. By analyzing the relationships between various investigated parameters, a Generic Optimal Cooling Rate Chart (GOCRC) is developed.

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