Biochimica et Biophysica Acta, 465 (1977) 179—190

BBA 77623

EFFECT OF SOLUTION NON-IDEALITY ON ERYTHROCYTE VOLUME REGULATION

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(Received September 9th, 1976)

Summary

A non-ideal, hydrated, non-dilute pseudo-binary salt-protein-water solution model of the erythrocyte intracellular solution is presented to describe the osmotic behavior of human erythrocytes. Existing experimental activity data for salts and proteins in aqueous solutions are used to formulate van Laar type expressions for the solvent and solute activity coefficients. Reasonable estimates can therefore be made of the non-ideality of the erythrocyte intracellular solution over a wide range of osmolalities. Solution non-ideality is shown to affect significantly the degree of solute polarization within the erythrocyte intracellular solution during freezing. However, the non-ideality has very little effect upon the amount of water retained within erythrocytes cooled at sub-zero temperatures.

Introduction

In two previous publications [1,2], the kinetics of water transport within erythrocytes during cooling at sub-zero temperatures were analyzed. The analyses assumed that the erythrocyte intracellular solution can be considered as an ideal, hydrated, non-dilute pseudo-binary solution of salts, proteins, and water. In the present work we shall extend our studies of the effects of hydration and solute polarization on the water content of human erythrocytes to include the effects of solution non-ideality.

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Activity coefficients

The activity a_i of the ith species in a multicomponent solution is defined as

$$a_i = f_i x_i \tag{1}$$

where f_i is the activity coefficient defined with respect to an ideal dilute solution such that

$$\begin{aligned} &f_w \to 1 \text{ as } x_w \to 1 \text{ (solvent)} \\ &f_{i \neq w} \to 1 \text{ as } x_{i \neq w} \to 0 \text{ (solute)} \end{aligned} \tag{2}$$

The activity of the solvent a_w in a multicomponent solution can also be defined as (Katchalsky and Alexandrowicz ref. 3)

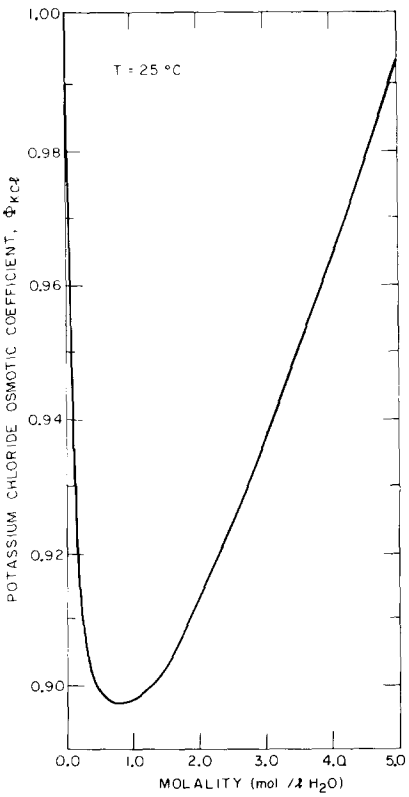
$$\ln a_w = -\frac{18}{10^3} \sum_{i \neq w} (\Phi_i \nu_i m_i) \tag{3}$$

where Φ_i is the osmotic coefficient and m_i is the molality of species i in solvent w (mol/ℓ H_2O). Consequently, for the erythrocyte salt-protein-water intracellular solution, Eqn. 3 takes the form

$$\ln a_w = -\frac{18}{10^3} (\Phi_s \nu_s m_s + \Phi_p m_p) \tag{4}$$

The osmotic coefficients for various common salts (NaCl, KCl, $CaCl_2$, etc.) in water are well documented for temperatures above the freezing point of the aqueous solution [4]. For the hydration numbers and apparent molar volumes of the salts within the erythrocyte intracellular solution, we shall assume [1] that Φ_S can be approximated by the osmotic coefficient of potassium chloride in water at 25°C. As shown in Fig. 1, the osmotic coefficient of KCl first decreases from 1.0 at $m = 0$, reaching a minimum of 0.9870 at $m_{KCl} \approx 0.8$ mol/ℓ H_2O ($m_{KCl)o} = 0.150$ mol/ℓ H_2O) and then increases approximately linearly with concentration to a value of 0.9883 at $m = 4.8$ mol/ℓ H_2O. This type of positive departure from ideality ($\Phi < 1$) is common for electrolytes. The infinite negative gradient of the curve as zero concentration is approached is a consequence of the long range electrostatic attractions and repulsions obeying the inverse square law, while the more or less linear rise at higher concentrations is due to short range Van der Waals forces, ion-dipole interactions, etc.

Unfortunately, data for the osmotic coefficients of hemoglobin and other cell proteins are less well documented. Adair [5] measured Φ_{Hb} in dilute Hb-water solutions ($m_{Hb} < 7.71$ mmol/ℓ H_2O) at 0°C; McConaghey and Maizels [6] measured Φ_{Hb} in erythrocytes suspended in NaCl solutions at somewhat higher concentrations (5.3—15.9 mmol/ℓ H_2O) and at 20°C. The data of both Adair and McConaghey and Maizels are plotted in Fig. 2. Comparison of Φ_{Hb} with Φ_{KCl} indicates that the osmotic coefficient of hemoglobin exhibits negative deviation from ideality ($\Phi > 1$) and increases much more rapidly with increasing concentration than does the osmotic coefficient of potassium chloride. This large rise in the osmotic pressure of a protein with concentration is attributed to the marked increase in the entropy of mixing which arises from the dispro-



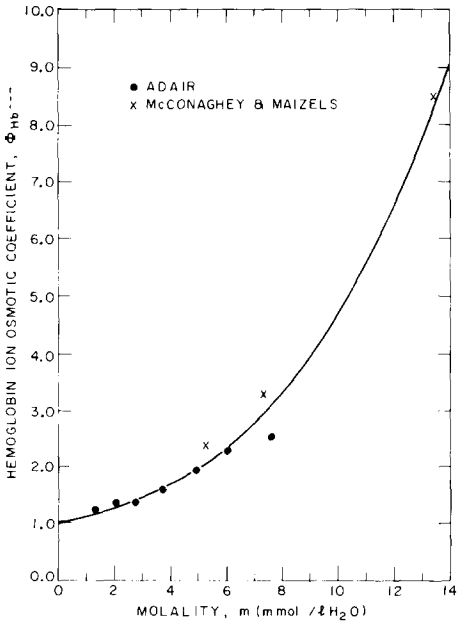


Fig. 1. Potassium chloride osmotic coefficient. See Robinson and Stokes ref. 4.

Fig. 2. Hemoglobin ion osmotic coefficient. See Adair ref. 5 and McConaghey and Maizels ref. 6.

portion between the small molecules of the solvent and the large molecules of the protein solute ($\bar{v}^{h}_{Hb}/\bar{v}_{w} \approx 4000$) [7].

On the basis of these data for the osmotic coefficients of KCl and Hb and on the basis of the composition of the pseudo-binary erythrocyte intracellular solution under isotonic conditions listed in Table I [1], the activity coefficients for the solvent w and the fictitious solute species m were derived using Eqns. 1 and 4 for $0.36 < \phi_w < 0.6193$ (i.e. a concentration range of $7.22 \cdot 10^{-3}$ to $15.9 \cdot 10^{-3}$ mol/ℓ H_2O for the protein and 0.150 to 0.330 mol/ℓ H_2O for the salt). In our opinion, however, it would be an error to simply extrapolate the data for the hemoglobin osmotic coefficient to higher concentrations ($m_{Hb} > 15.9$ mmol/ℓ H_2O) since even a linear extrapolation would yield seemingly unrealistically high values for Φ_P at protein concentrations of 20—40 mmol/ℓ H_2O. A simple extrapolation would also fail to take into account any possible changes in the behavior of the solution in the limit $\phi_w \rightarrow 0$ as all of the free water leaves the cell. In order to extend the existing dilute solution activity data to very concentrated solutions, let us briefly consider the various solution theories that have been proposed.

Most solution theories tend to explain solution non-ideality in terms of physical (Coulomb, Van der Waals, etc.) intermolecular forces. These theories

TABLE I

PSEUDO-BINARY SOLUTION PROPERTIES OF MODEL ERYTHROCYTE

Species	ν	$\bar{v}$ (cm^3/mol)	$\bar{v}_3^h$ (cm^3/mol)	h (mol H_2O/mol)	N_o (mol)	Isotonic cell			Dehydrated cell		
						m(mmol/l H_2O)	x^h	ϕ	m(mmol/l H_2O)	x^h	ϕ
Water	1.0	18.0	18.0	—	$4.00 \cdot 10^{-12}$	—	0.99341	0.6193	—	0.00	0.00
Salt	2.0	27.5	45.5	1.0	$1.08 \cdot 10^{-14}$	150.0	$6.437 \cdot 10^{-3}$	$5.07 \cdot 10^{-3}$	902.0	0.9765	0.0133
Protein	1.0	$5.0 \cdot 10^4$	$7.0 \cdot 10^4$	1260.0	$5.20 \cdot 10^{-16}$	7.22	$1.550 \cdot 10^{-4}$	0.3756	43.4	0.0235	0.9867
Solute mixture *	1.0	—	$1.7 \cdot 10^3$	30.1	$2.21 \cdot 10^{-14}$	157.2	$6.592 \cdot 10^{-3}$	0.3807	945.4	1.00	1.00

* $N_m = N_p + \upsilon_s N_s$; $\bar{v}_m^h = (N_p \bar{v}_p^h + N_s \bar{v}_s^h)/N_m$; $h_m = (h_p N_p + h_s N_s)/N_m$.

(e.g. Debye-Hückel electrolyte solution theory, Scatchard-Hildebrand regular solution theory, Flory-Huggins theory) relate the activity coefficients to physical quantities which reflect the size of the molecules and the physical forces operating between them. An alternate approach to the study of solution properties is based on a rather different premise, viz., that molecules in a liquid solution interact with each other to form new chemical species and that solution non-ideality, therefore, is a consequence of chemical reactions [8].

The various physical theories of solutions and the chemical solution theory of Dolezalek [8] are extreme, one-sided statements of what is now believed to be the actual situation. In certain limiting cases each of these points of view provides a satisfactory approximation. When forces between the molecules are weak, no new stable chemical species are formed and the physical theory applies; on the other hand, when forces between molecules are strong, these forces result in the formation of chemical bonds and since the energies for chemical bond formation are significantly larger than those corresponding to Van der Waals forces, the chemical theory applies. In general, both physical and chemical forces should be taken into account. This is what we shall attempt to do for the case of the erythrocyte intracellular solution.

It is difficult to formulate a theory which takes account of both physical and chemical effects without introducing involved algebra and, what is worse, a large number of adjustable parameters. Consequently, we shall follow the lead of Harris [9] in assuming that the activity coefficients of our two solution species (solvent w and hydrated fictitious solute m) can be described by an equation of the van Laar type (see Prausnitz ref. 10):

$$\ln f_w^h = \frac{\alpha}{\left(1 + \frac{\alpha}{\beta}(x_w^h/x_m^h)\right)^2} \tag{5a}$$

$$\ln f_m^h = \frac{\beta}{\left(1 + \frac{\beta}{\alpha}(x_m^h/x_w^h)\right)^2} - \beta \tag{5b}$$

where we have defined f_i^h to be the activity coefficients of species i computed on a hydrated basis (see Levin et al. ref. 1):

$$a_i = f_i^h x_i^h \qquad i = w,m \tag{6}$$

The quantities x_i^h are the mole fractions of species i computed on a hydrated basis [1]:

$$x_w^h = \frac{N_w - h_m N_m}{N_w + [1 - (h_m/\nu_m)]\nu_m N_m}$$

$$x_m^h = \frac{\nu_m N_m}{N_w + [1 - (h_m/\nu_m)]\nu_m N_m} \tag{7}$$

N_m and h_m are respectively, the number of moles and hydration number of the fictitious solution species m considered to be present within the cell.

The correlation of data for our pseudo-binary system is accomplished most

easily by plotting the activity data so that straight lines result. Eqn. 5a may be written:

$$(\ln f_w^h)^{-1/2} = (\alpha^{1/2}/\beta) \cdot (x_w^h/x_m^h) + (1/\alpha^{1/2}) \tag{8}$$

Thus, a plot of $(\ln f_w^h)^{-1/2}$ versus x_w^h/x_m^h should yield a straight line having a slope $\alpha^{1/2}/\beta$ and an intercept $1/\alpha^{1/2}$. The activity data for our erythrocyte intracellular solution are plotted in Fig. 3 in just this manner and a straight line does indeed result. A "least squares fit" of the data plotted in Fig. 3 yields values for the slope of 0.138 ± 0.002 and for the intercept of 7.29 ± 0.06 at the 5% confidence level. On the basis of these results, we feel that the activity coefficients of w and m in our pseudo-binary intracellular erythrocyte solution can be adequately represented even at high solute concentrations by the following relations:

$$\ln f_w^h = \frac{1.88 \cdot 10^{-2}}{[1 + 1.89 \cdot 10^{-2}(x_w^h/x_m^h)]^2}$$
$$\ln f_m^h = \frac{0.994}{[1 + 52.9(x_m^h/x_w^h)]^2} - 0.994 \tag{9}$$

Note that Eqn. 5 is identical in form to the "so-called" van Laar equation [10] except that the effects of temperature and pressure have been omitted. According to van Laar's original derivation [11] the constants α and β of Eqn. 5 are not dependent upon composition but instead are functions of temperature and pressure. Since the effects of pressure on liquid-phase properties is usually small (except at high pressures and at conditions near the critical state) the pressure dependence of the constants can usually be neglected; however, the temperature-dependence is often not negligible. Since in our case, the experimental data are insufficient to specify the temperature-dependence of the activity coefficients, either one of two simplifying assumptions can be made. The first one is to assume that at constant composition the activity

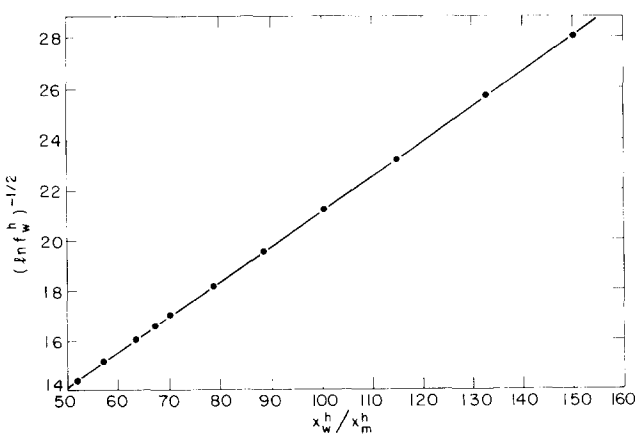


Fig. 3. Van Laar representation of the water activity for the hydrated, non-dilute pseudo-binary salt-protein-water erythrocyte intracellular solution.

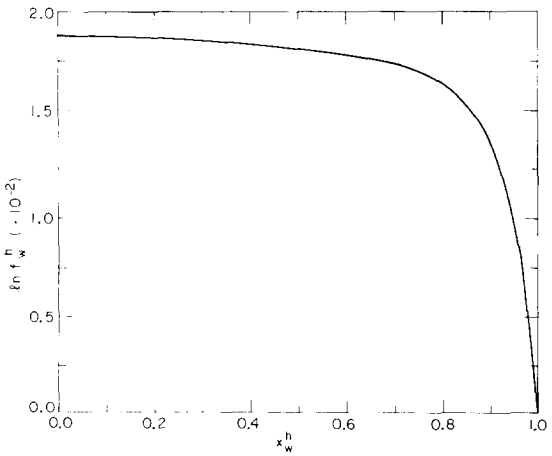


Fig. 4. Mole fraction dependence of the water activity coefficient for the hydrated, non-dilute pseudo-binary salt-protein-water erythrocyte intracellular solution.

coefficients are invariant with temperature; the second is to assume that at constant composition ln f is proportional to the reciprocal of the absolute temperature. The first assumption is equivalent to assuming that the solution is athermal ($\Delta H_{\text{mixing}} = 0$), and the second is equivalent to assuming that the solution is regular ($\Delta S^{\text{E}}_{\text{mixing}} = 0$). Actual solutions, of course, are neither athermal nor regular. Because of the large differences in the size and shape of the solvent (water) and solute (salt and protein) molecules of the erythrocyte intracellular solution, we shall assume that our pseudo-binary solution is athermal with a non-zero entropy of mixing. Consequently, the values of $\alpha = 1.88 \cdot 10^{-2}$ and

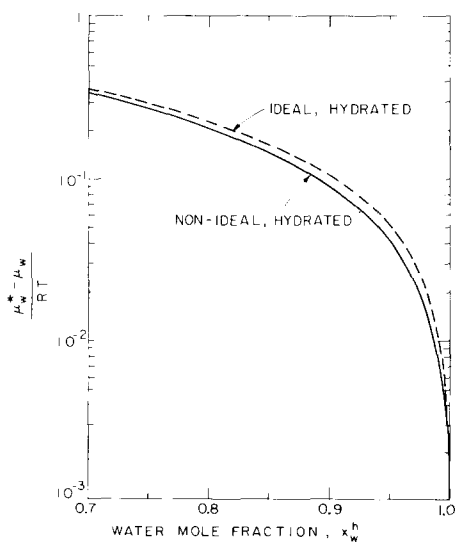


Fig. 5. Comparison of the mole fraction dependence of the chemical potential of an ideal, hydrated and a non-ideal, hydrated non-dilute pseudo-binary erythrocyte intracellular solution.

$\beta = 0.994$ used in Eqn. 9 shall be assumed to be independent of temperature.

The logarithm of the water activity coefficient ($\ln f_w^h$) is plotted as a function of the hydrated water mole fraction (x_w^h) in Fig. 4. Note that $\ln f_w^h$ increases very rapidly from 0 to $1.63 \cdot 10^{-2}$ in the region $1.00 > x_w^h > 0.8$. This increase can be attributed to the large difference in the apparent molar volumes of the solvent and solute species which causes the volume fraction of water to vary from 1.00 to ≈ 0.05 in the same interval. The effect of this concentration dependence of $\ln f_w^h$ on the water activity can be seen in Fig. 5. Again the major effects of assuming a non-ideal solution ($f_w^h \neq 1$) manifest themselves in the region $1.00 > x_w^h > 0.80$ where $1.00 > \phi_w > 0.05$.

Application of the analysis

Using the transport equations developed by Levin et al. [12] for the movement of solute and solvent within the erythrocyte intracellular solution and through the erythrocyte membrane, we have applied the analysis to the problem of determining the behavior of human erythrocytes during freezing. This situation is one of considerable clinical importance in the reversible freezing of blood for long-term storage.

Clinical experience has shown that for human red blood cells suspended in an isotonic saline solution (0.154 mol NaCl/ℓ H_2O), ice tends to form extracellularly once the freezing point of the solution is reached. As water is removed from solution in the form of ice, the transmembrane chemical equilibrium is upset and the cell responds by expressing water across the membrane. As the water leaves the cell, the cell volume decreases and the cell membrane is displaced inwardly. Our experience [13] has shown that under these conditions, we can assume that:

(1) The temperature of the cell suspension is uniform. (2) The composition of the liquid phase of the extracellular medium is uniform with no polarization of solutes taking place at either the external surface of the cell membrane or at the liquid-solid interfaces. (3) The red blood cells are suspended in an initially isotonic sodium chloride aqueous solution. (4) Solid phase of pure ice forms only in the extracellular medium. (5) Chemical equilibrium prevails at all times in the extracellular medium. (6) The intracellular solution is a non-ideal, hydrated, non-dilute, pseudo-binary salt-protein-water solution initially of uniform composition. (7) The cell membrane is permeable to water only.

In Fig. 6 the loci of thermodynamic states of the intracellular solution at the internal surface of the cell membrane for both ideal and non-ideal intracellular solution cases are shown for erythrocytes cooled at $B = -5000°C/min$. The overall shape of the curves in Fig. 6 is essentially the same; however, quantitatively, the loci of states for the ideal and non-ideal intracellular solution cases are quite different, especially at the higher temperatures. The difference can be attributed directly to the fact that $f_w^h \neq 1$ but varies with composition for the case of the non-ideal intracellular solution. Since $\ln f_w^h = 1.27 \cdot 10^{-3}$ ($f_w^h = 1.0013$) under isotonic conditions, the temperature at which the loci of thermodynamic states of the intracellular solution intersect the two phase NaCl-H_2O equilibrium curve is even different for the two cases. For the ideal intracellular solution case, the freezing point of the intracellular solution is

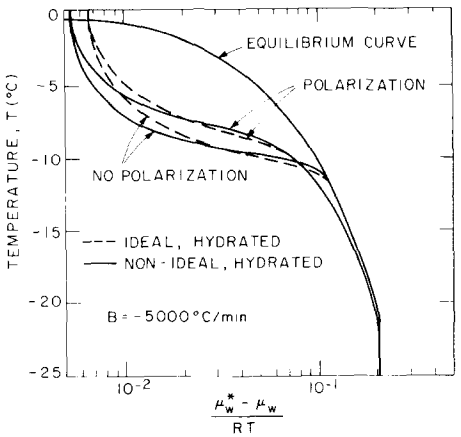


Fig. 6. Effect of solution non-ideality on the thermodynamic states of the hydrated pseudo-binary intracellular solution of erythrocytes cooled at 5000°C/min. Locus of states of extracellular solution coincides with two-phase equilibrium states for all cooling rates.

depressed 0.682 K whereas for the non-ideal intracellular solution case the freezing point is depressed only 0.551 K (see Fig. 6).

According to Fig. 6, at the higher temperatures, $\Delta\mu_w|_{\text{non-ideal}} > \Delta\mu_w|_{\text{ideal}}$, an occurrence which is a direct result of the fact that the erythrocyte intracellular solution exhibits positive deviation from ideality, i.e. $f > 1$. Consequently, one would expect that in this temperature range the magnitude of the water volume flux should be greater for the case of a non-ideal intracellular solution than for the case of an ideal intracellular solution. However, this effect is small. For B = —5000°C/min,

$$1.005 < \frac{\overline{v}_w \cdot J_w\big|_{\text{max, non-ideal}}}{\overline{v}_w \cdot J_w\big|_{\text{max, ideal}}} < 1.05$$

Nevertheless, since at any temperature $D^v_{\text{non-ideal}}/D^v_{\text{ideal}} = \partial \ln a_m/\partial \ln x_m < 1$ (see Levin et al. ref. 13), more solute will build up at the internal solution of the cell membrane for the non-ideal intracellular solution case than for the ideal intracellular solution case (see Fig. 7). For a cooling rate of 5000°C/min, $\phi_{m/e}/\phi_{m/\ell}|_{max} = 1.50$ at $T = -8.29°C$ for the non-ideal case whereas $\phi_{m/e}/\phi_{m/\ell}|_{max} = 1.39$ at $T = -9.00°C$ for the ideal case.

Although the non-ideality of the erythrocyte intracellular solution does significantly affect the degree of solute polarization, it has very little effect upon the amount of water retained within erythrocytes being cooled at sub-zero temperatures. This can be seen in Fig. 8 where the amount of water retained within erythrocytes cooled at 5000°C/min is plotted as a function of temperature for ideal and non-ideal intracellular solutions and polarized and unpolarized cases. In general, for the unpolarized case the erythrocyte water content will be slightly smaller for the non-ideal intracellular solution case than for the ideal intracellular solution case because the magnitude of the water volume flux is slightly higher. However, for the polarized case, the water content of erythrocytes will be slightly larger for the non-ideal intracellular solution case because the degree of solute polarization is greater. Finally, it should be noted that

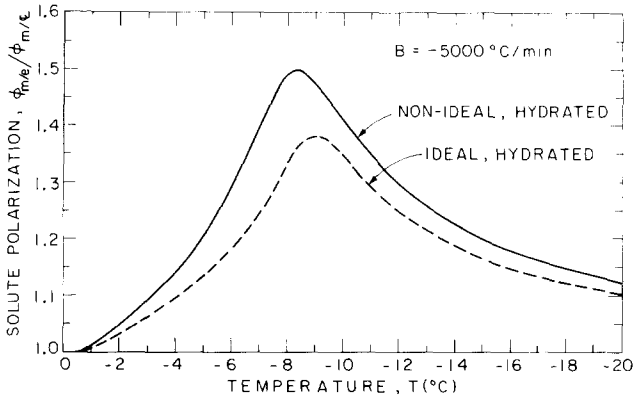


Fig. 7. Effect of solution non-ideality on the degree of solute polarization, $\phi_{m/e}/\phi_{m/\ell}$, within the pseudo-binary intracellular solution of erythrocytes cooled at 5000° C/min.

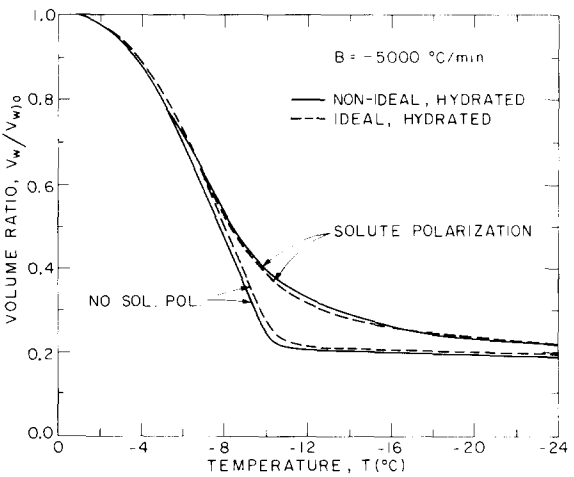


Fig. 8. Effect of solution non-ideality on the volume of intracellular water retained within erythrocytes cooled at different rates for the pseudo-binary intracellular solution case.

because of the positive deviation from ideality of the erythrocyte intracellular solution, the value of the water content for erythrocytes in an aqueous NaCl solution will be lower as the cell approaches equilibrium for the case of a non-ideal intracellular solution: $V_w/V_{w/o}|_{\text{non-ideal}} \rightarrow 0.1887$ ($\phi_m|_{\text{non-ideal}} \rightarrow 0.9584$) as $\Delta\mu_w \rightarrow 0$, than for the case of an ideal intracellular solution, $V_w/V_{w/o}|_{\text{ideal}} \rightarrow 0.1907$ ($\phi_m|_{\text{ideal}} \rightarrow 0.9550$) as $\Delta\mu_w \rightarrow 0$.

Glossary

a = activity
B = cooling rate
c = molar concentration
D^V = volume diffusivity
f = activity coefficient

H = enthalpy
J = flux
m = molality
N = number of molecules
S = entropy
s = solute
T = temperature (K)
T_o = freezing point (273.15 K)
t = time
V = volume
V_c = cell volume
$\bar{v}$ = apparent molar volume
w = water
x = mole fraction
α = van Laar parameter
β = van Laar parameter
ν = number of species per molecule
Φ = osmotic coefficient
ϕ = volume fraction
μ = chemical potential

Subscript
i = ith species
m = solute mixture
o = initial or isotonic
P = protein salt
S = inorganic salt
s = solute
w = water

Superscript
E = excess function
h = hydrated
I = intracellular
O = extracellular

Acknowledgement

This work was supported in part by the Biomaterials Science Program Project Grant H05 P01 HL-14322 funded by the National Heart and Lung Institute under the Harvard-MIT Program in Health Sciences and Technology.

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