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Comparison of non-ideal solution theories for multi-solute solutions in cryobiology and tabulation of required coefficients ☆

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ABSTRACT

Thermodynamic solution theories allow the prediction of chemical potentials in solutions of known composition. In cryobiology, such models are a critical component of many mathematical models that are used to simulate the biophysical processes occurring in cells and tissues during cryopreservation. A number of solution theories, both thermodynamically ideal and non-ideal, have been proposed for use with cryobiological solutions. In this work, we have evaluated two non-ideal solution theories for predicting water chemical potential (*i.e.* osmolality) in multi-solute solutions relevant to cryobiology: the Elliott et al. form of the multi-solute osmotic virial equation, and the Kleinhans and Mazur freezing point summation model. These two solution theories require fitting to only single-solute data, although they can make predictions in multi-solute solutions. The predictions of these non-ideal solution theories were compared to predictions made using ideal dilute assumptions and to available literature multi-solute experimental osmometric data. A single, consistent set of literature single-solute solution data was used to fit for the required solute-specific coefficients for each of the non-ideal models. Our results indicate that the two non-ideal solution theories have similar overall performance, and both give more accurate predictions than ideal models. These results can be used to select between the non-ideal models for a specific multi-solute solution, and the updated coefficients provided in this work can be used to make the desired predictions.

Introduction

Many of the mathematical models that are used to simulate cryopreservation protocols [1,2,15,25,26,31,34,35,44,54,59,60,68] rely on the ability to accurately predict thermodynamic solution behavior, since important processes such as water and solute transport and ice formation are ultimately dictated by differences in chemical potential. As a consequence, it is important to give some thought to the choice of the solution theories that are used to calculate these chemical potentials. This article examines and evaluates some of the available theories for predicting water (*i.e.* solvent) chemical potential, in particular those that do not depend on multi-solute solution data.

In cryobiology, water chemical potential is often expressed in terms of its composition dependence, osmolality [3,11,14,15,21,55,56,74], or in terms of the related properties freezing point depression [3,14–16,21,38,50–52,55,74–76] and osmotic pressure [37,44,55,73]. Freezing point depression and osmotic pressure are physically measurable solution properties, and the relationships between them and osmolality (described below in Eqs. (2) and (3) and in Eq. (4), respectively) allow one to experimentally obtain values for the osmolality of a solution. Solution osmolality can also be related to other measurable properties, including vapor pressure [23,67] and, for polymers, light scattering (based on index of refraction) [22,28,29,36,58]. Such relationships form the basis of osmometry, and allow one to measure the osmolality of any solution of interest. However, for the purposes of modeling cryopreservation processes, measuring the osmolality of every solution of interest is not feasible (*e.g.* solution compositions change constantly as ice forms, or when cryoprotectants are added), nor is it always possible (*e.g.* intracellular solutions are not accessible for instantaneous measurement). As such, the ability to accurately predict the solution osmolality is essential for cryobiological models where this property is an input.

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By their nature, cryobiological solutions contain diverse solutes ranging from salts and cryoprotectants to proteins and other macromolecules, often at high concentrations—even those solutions that are relatively dilute at room temperature become highly concentrated when frozen. As a result, cryobiological solutions are generally thermodynamically non-ideal. Although this non-ideality can be ignored and an ideal dilute solution theory can be used to model the solution behavior [18,25,26,31–35,44,68], doing so can introduce significant errors in the predictions of chemical potential [14,55,56]. Accordingly, there are a number of solution theories available in the literature which account for solution non-ideality and have been demonstrated to accurately model the osmolality of multi-solute solutions of cryobiological interest [3,7,14,16,38,50–52,55,56,76]. However, the majority of these solution theories depend on fitting to multi-solute data, meaning that every solution system (*i.e.* combination of solutes) of interest must be fit independently prior to being modeled [3,16,50–52,76]. Considering the vast range of possible solution systems that are relevant in cryobiology (*e.g.* cytoplasm, plasma and interstitial fluids, multi-cryoprotectant vitrification cocktails [17,27,46]) and the challenges inherent to the measurement of multi-solute phase diagrams (*e.g.* the number of measurements required for a given compositional resolution increases exponentially with the number of solutes present in solution) [38], this type of approach is not practical for general modeling applications. Alternatively, there are at least two solution theories available which allow the prediction of osmolality in non-ideal multi-solute solutions using only single-solute (*i.e.* binary solution) data: the form of the multi-solute osmotic virial equation developed by Elliott et al. [7,14,15,55,56], and the freezing point summation model of Kleinhans and Mazur [38]. The primary aim of this work is to compare predictions of multi-solute solution osmolality made with these two non-ideal solution theories to available experimental data, to one another, and to ideal dilute model predictions. This work expands upon earlier comparisons [14,55], employing a larger set of literature data, and addressing statistical and thermodynamic issues in the previous studies.

Multi-solute solution theories used in cryobiology

Solution thermodynamic properties

As mentioned above, osmolality, freezing point depression, and osmotic pressure are all related to one another and, ultimately, to water chemical potential. As these properties will be used interchangeably throughout this paper, we have summarized the relationships between them here. Osmolality, π, is mathematically defined as [14]

$$\pi = -\frac{\mu_1 - \mu_1^o}{\mathrm{RTM}_1}, \tag{1}$$

where μ_1 is the chemical potential of water, μ_1^o is the chemical potential of pure water, R is the universal gas constant, T is absolute temperature (in Kelvin), and M_1 is the molar mass of water (note that the subscript “1” is typically reserved for the solvent—in this case, water). Freezing point depression, ΔT_{m}, and osmolality are related by [55]

$$\Delta T_{\mathrm{m}} = T_{\mathrm{m}}^o - T_{\mathrm{m}} = \frac{RT_{\mathrm{m}}^o \pi \left[M_1/\overline{\Delta s_{f1}^\circ}\right]}{1 + R\pi\left[M_1/\overline{\Delta s_{f1}^\circ}\right]}, \tag{2}$$

or, equivalently

$$\pi = \frac{\Delta T_{\mathrm{m}}}{RT_{\mathrm{m}}[M_1/\overline{\Delta s_{f1}^\circ}]} = \frac{T_{\mathrm{m}}^o - T_{\mathrm{m}}}{RT_{\mathrm{m}}\left[M_1/\overline{\Delta s_{f1}^\circ}\right]}, \tag{3}$$

where T_{m} is the absolute freezing point of the solution, T_{m}^o is the absolute freezing point of pure water, and $\overline{\Delta s_{f1}^\circ}$ is the standard molar entropy change of fusion of water. Eq. (3) is commonly linearized as $\pi = \Delta T_m/1.86$; however, this linearization introduces considerable error [55] and will not be used here. Osmotic pressure, Π, is related to osmolality by [55]

$$\Pi = RT\rho_1\pi, \tag{4}$$

where ρ_1 is the density of water. The values and units of the constants in Eqs. (1)–(4) are contained in Table 1.

Elliott et al. multi-solute osmotic virial equation

The Elliott et al. multi-solute osmotic virial equation is based on the osmotic virial equation of McMillan and Mayer [45], an equation of state in which the osmolality is represented as a polynomial in terms of solute concentration. Depending on the underlying theoretical assumptions, different units of concentration can be used, giving two distinct thermodynamic models [14]. In terms of molal concentration or molality (*i.e.* moles of solute per kg of solvent), following Landau and Lifshitz solution theory [42], the single-solute osmotic virial equation for solute i is [14,45]

$$\pi = m_i + B_{ii}m_i^2 + C_{iii}m_i^3 + \ldots, \tag{5}$$

where m_i is the molality of solute i (in moles of solute/kg of water), and B_{ii} and C_{iii} are the second and third molality-based osmotic virial coefficients of solute i, respectively (in [moles of solute/kg of water]$^{-1}$ and [moles of solute/kg of water]$^{-2}$, respectively). Alternatively, in terms of solute concentration in mole fraction (*i.e.* moles of solute per total moles of all species), per regular solution theory [53], the single-solute osmotic virial equation for solute i is [45,55]

$$\tilde{\pi} = x_i + B_{ii}^* x_i^2 + C_{iii}^* x_i^3 + \ldots, \tag{6}$$

where $\tilde{\pi}$ is osmole fraction (unitless), x_i is the mole fraction of solute i, and B_{ii}^* and C_{iii}^* are the second and third mole fraction-based osmotic virial coefficients of solute i, respectively (unitless). Osmole fraction is a rarely-used alternative form of osmolality, defined as [14]

$$\tilde{\pi} = -\frac{\mu_1 - \mu_1^o}{RT}. \tag{7}$$

Comparing Eqs. (1) and (7), osmolality and osmole fraction are related by

$$\tilde{\pi} = M_1\pi. \tag{8}$$

The osmotic virial coefficients in Eqs. (5) and (6) account for increasing orders of interaction between molecules of solute i: the second osmotic virial coefficient represents interactions between two solute i molecules, the third osmotic virial coefficient represents interactions between three solute i molecules, and so forth. As such, these coefficients represent the non-ideality of the solute—if they are all zero, solute i is thermodynamically ideal. For electrolyte solutes, solute concentration must be multiplied by an additional parameter, the dissociation constant [56]

$$\pi = k_i m_i + B_{ii}(k_i m_i)^2 + C_{iii}(k_i m_i)^3 + \ldots, \tag{9}$$

$$\tilde{\pi} = k_i^* x_i + B_{ii}^*(k_i^* x_i)^2 + C_{iii}^*(k_i^* x_i)^3 + \ldots, \tag{10}$$

Table 1
Values and units of the constants in Eqs. (1)–(4) [6].

Constant	Value
R	8.314 J/(mol K) = 8.314 Pa m^3/(mol K)
M_1	1.802×10^{-2} kg/mol
T_m^o	273.15 K
$\overline{\Delta s_{f1}^\circ}$	22.00 J/(mol K)
ρ_1	997 kg/m^3