

## A. Water Transport in Mitochondria

Suppose we set up our experiment so that water moves from one side of the membrane to the other. The chemical potential of water,  $\mu_w$ , may be written

$$\mu_w = \mu_w^0 + RT \ln a_w \quad (1)$$

where  $\mu_w^0$  is the chemical potential of pure water and  $a_w$  is the thermodynamic activity of water in the solution. Note that this definition differs from solute chemical potentials such that  $a_w = 1.0$  for pure water. The free energy difference between the two compartments is therefore given by

$$\Delta\mu_w = \mu_{w2} - \mu_{w1} = RT \ln (a_{w2} / a_{w1}) \quad (2)$$

We know from this equation that water will only flow from side 1 to side 2 if  $a_{w2} < a_{w1}$ ; i.e., if  $\Delta\mu_w < 0$ .

We obtain the same result if we use Fick's Law. Because water is a nonelectrolyte, we may write:

$$J_w = P_w (a_{w1} - a_{w2}) \quad (3)$$

where  $P_w$  is the permeability constant of the membrane for water. This equation tells us the same thing - water will flow until  $a_{w2} = a_{w1}$ .

Water movement across the inner membrane is very rapid, and we may therefore assume that *mitochondria are in a state of partial equilibrium with respect to the process of water transport*. This means that anything that promotes water movement via Eq. 3

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has the potential to perturb the structure of mitochondria and thereby interfere with their function. The questions that we will address include: how do changes in the activity of water affect mitochondria? How are these changes effected? How do we measure water activity? This will require some basic thermodynamics.

## B. Colligative Properties of Solutions

This section is to show you from whence the concept of “colligative properties” arises.

### 1. Molarity, molality, and mole fraction

**Molarity (C) - Units: M (mol/liter):** Let  $w$  = weight of solute and  $M$  its molecular weight.

$$C = (w / M) \times (1000/V) \quad (1)$$

where  $V$  is *final* volume of the solution in milliliters. This is the quantity most convenient and common to use.

**Molality (m) - Units: mol/kg solvent:** dissolve solute in  $W_1$  grams of solvent.

$$m = (w / M) \times (1000/W_1) \quad (2)$$

Note that in all aqueous solutions,  $m(\text{water}) = 1000/M_1 \sim 55.6$  molal, where  $M_1$  is mw of water. This is the proper thermodynamic variable to use in studies of water activity.

**Mole fraction ( $N_i$ ):** Very useful for development of thermodynamic quantities.

$$N_i = x_i / \sum x_j \quad (3)$$

Note from Eq 2 that this can also be written

$$N_i = m_i / \sum m_j \quad (4)$$

(As a matter of interest, one can convert concentration to molal, if you know its composition and the density of the solution,  $\rho$ . Clearly, the following is true:

$$m_i = c_i / c_w$$

where  $c_w$  is the concentration of *water* in the solution (kg water/L solution). It is easy to show that

$$c_w = \rho - \sum c_j M_j / 1000$$

Note that the first term ( $\rho$ ) is the total weight/liter of the solution and the second term is the dry weight/liter.)

## **2. Vapor Pressure of an Ideal Solution**

The vapor pressure of a solution,  $p$ , is the total pressure of the gases in equilibrium with the liquid solution. For ideal gases, the total pressure,  $P$ , is the sum of the vapor pressures of the several volatile components (Dalton's Law):

$$P = \sum p_i \tag{5}$$

Species  $i$  in the gas phase, with partial pressure,  $p_i$ , is in equilibrium species  $i$  in the ideal liquid solution, with mole fraction,  $N_i$ . At constant  $T$ ,  $p_i$  is given by (Raoult's Law):

$$p_i = N_i p_i^0 \tag{6}$$

where  $p_i^0$  is the vapor pressure of pure  $i$  at the same  $T$ .

## **3. Colligative Properties of a Solution**

Consider a 2 component system. Component 1 is water and component 2 is a non-volatile solute, such as sucrose. Sucrose effectively has zero vapor pressure, and  $p_2 = 0$ . If only water is volatile, then its vapor pressure is the total pressure

$$p_1 = P \tag{7}$$

and, from Raoult's law,

$$p_1 = N_1 p_1^{\circ} \quad (8)$$

It is clear from the definition of mole fraction (Eq. 3) that  $N_1 + N_2 = 1$ . Use this to eliminate  $N_1$  from Eq. 8, then

$$p_1 = p_1^{\circ} - N_2 p_1^{\circ}, \text{ and}$$

$$N_2 = (p_1^{\circ} - p_1) / p_1^{\circ} \quad (9)$$

Eq 9 says that vapor pressure lowering ( $p_1^{\circ} - p_1$ ) is proportional to the fraction of nonvolatile molecules. There are three important aspects of this equation:

(a) In the example used, we presumably weighed the sucrose and the water before we mixed them. From algebraic rearrangement of Eq 9, we obtain

$$M_2 = (W_1/M_1 w_2) \times (1 - 1/N_2) \quad (10)$$

Eq. 10 says that, once we have obtained  $N_2$  from the vapor pressure lowering experiment, we can determine the molecular weight of the solute. Many molecular weights were obtained in this way.

(b) In the derivation above, I used an example of a 2-component system. You may rederive it with a number of nonvolatile species and one volatile species, "1". Note that the result (Eq 9) is the same - except that  $N_2$  now refers to the mole fraction of *all* non-volatile molecules present. *A property that varies according to the number of particles without regard to their kind is called a **colligative property**.*

(c) The effect of adding a solute to a pure solvent is to lower the vapor pressure of the solvent. It can be shown that it lowers the thermodynamic *activity* of the solvent. Other colligative properties are very important in biology: osmolality (discussed in the next

section) is a measure of the thermodynamic *activity* of water. Freezing point depression by solutes is colligative, and we can use that to measure the osmolality of a solution.

### C. Osmolality of a Solution

#### 1. Osmolality and the Osmotic Coefficient of a Solution

The free energy of a solution at constant temperature and pressure is a linear homogeneous function of the number of moles,  $N_j$ , of the system components.

Therefore, using Euler's Theorem,

$$G(T,P,N_1, \dots, N_n) = \sum N_j \mu_j \quad (1)$$

where

$$\mu_j \equiv \left( \frac{\partial G}{\partial N_j} \right)_{T,P,N_i} \quad (2)$$

Note that Eq. 1 just tells us that the total free energy of a solution equals the sum of the partial free energies of each component times the mole fraction of that component.

Differentiating both sides of Eq. (A1)

$$dG = \sum N_j d\mu_j + \sum \mu_j dN_j \quad (3)$$

but,

$$\sum \mu_j dN_j = \sum (\partial \mu_j / \partial N_j) dN_j = dG \quad (4)$$

Therefore, at constant temperature and pressure,

$$\sum N_j d\mu_j = 0 \quad (5)$$

This is the Gibbs-Duhem equation.

As, before, the chemical potential of the solvent (water),  $\mu_w$ , may be written

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$$\mu_w = \mu_w^0 + RT \ln a_w \quad (6)$$

The chemical potential of the solute,  $\mu_j$ , is

$$\mu_j = \mu_j^\ominus + RT \ln \gamma_j m_j \quad (7)$$

where  $\mu_j^\ominus$  is the solute chemical potential in a hypothetical ideal 1.0 molal solution, and  $\gamma_j$  is the molal activity coefficient. ( $\gamma_j m_j = a_j$ , where  $a_j$  is the thermodynamic activity of the solute. Note that  $a_j = m_j$  as the solute becomes very dilute, i.e., the solute approaches ideal behavior). Since  $m_j$  is the number of moles per kg of solvent, Eq. (5) may be written, for a single, non-dissociating solute:

$$\frac{1000}{M_w} d(\ln a_w) + m d(\ln \gamma m) = 0 \quad (8)$$

where  $M_w$  is the molecular weight of water. The osmotic coefficient,  $g$ , was introduced to enable integration of Eq. (8).  $g$  is *defined* by

$$\frac{-1000}{M_w} \ln a_w \equiv gm \equiv \Phi \quad (9)$$

The osmolality of the solution,  $\Phi$ , is simply equal to  $gm$ . Why do we use “osmolality”? The thermodynamic activity of water in cells,  $a_w$ , normally ranges between 0.990 and 0.997. The osmolality,  $\Phi$ , avoids the awkwardness of these numbers and leads to a range between 166 and 558 mosM. Note that osmolality, which can be measured by freezing point depression, osmotic pressure, or vapor pressure lowering, is a thermodynamically rigorous measure of water activity. It should be clear that ***osmolality depends solely on the activity of water in the system, and vice versa.*** This definition is sufficient for our purposes.

It should be evident from the derivation that the osmotic coefficient is a property of the *solution* and not of the solute. For a multicomponent solution, the osmolality may be written

$$\Phi = g \sum v_j m_j \quad (10)$$

where  $v_j$  is the number of particles into which solute  $j$  dissociates. To give the

equations a simpler form, I will use the convention

$$s \equiv \sum v_j m_j \quad (11)$$

where  $s$  is evidently the *ideal* osmolality. Accordingly, Eq. 10 becomes

$$\phi = g s \quad (12)$$

## 2. Measuring Osmolality

We generally measure osmolality of solutions by freezing point depression. The relationship (which can be derived from thermodynamics) is

$$\phi = \Delta T_f / K_f \quad (13)$$

where  $\Delta T_f$  is the freezing point depression caused by the presence of solute, and  $K_f$  is called the “cryoscopic constant”. For water,  $K_f = 1.862$ .

## 3. Determination of solute activity coefficients

Osmotic coefficients of solutes are measured in order to determine *solute activity coefficients*, and the following will show how this relationship is derived solely to illustrate this use.

Differentiating Eq. (9), substituting Eq. (8) and integrating over the composition variables leads to the relationship between the osmotic coefficient and activity coefficient of a nonelectrolyte:

$$(g - 1)m = \int m d(\ln \gamma) \quad (14)$$

In the case of a binary solution of nonelectrolyte and water, Eq. (14) may be integrated if the dependence of the activity coefficient on concentration is known. Note in thermodynamics that it can be shown that  $\gamma = 1$  for *ideal* solutions and that infinitely dilute solutions approach ideality. Therefore  $\gamma \rightarrow 1.0$  as  $m \rightarrow 0$ , and the solute activity coefficient of a binary mixture of nonelectrolyte and water may be expanded in a Taylor's series:

$$\ln \gamma \approx 2\alpha m + \dots \quad (15)$$

The osmotic coefficient of this binary solution is now readily obtained by integrating Eq. (14):

$$g = 1 + \alpha m \quad (16)$$

$g$  is experimentally accessible, because osmolality can be determined by freezing point depression or lowering of vapor pressure. Therefore, Eq. (16) provides a means to test the linear approximation of Eq. (15). Garlid (2000, water paper) has plotted data for sucrose; at concentrations less than 1.0 molal, the deviation from Eq. (16) is very small indeed.

## D. Osmometric Behavior of Mitochondria and of Polar Solutes

### *1 Osmotic behavior of polar solutes - introduction to the osmotic intercept*

Polar solutes exhibit non-ideal behavior in aqueous solutions, as illustrated by the osmotic behavior of sucrose and PEG400, plotted in Fig. 1 (Garlid, 2000). Both curves extrapolate to zero at infinite dilution, but deviate strongly from the line of ideal behavior ( $g = 1$ ). BSA exhibits similar behavior (not shown).

As shown 80 years ago by Scatchard (1921), nonideal behavior of polar solutes can be rationalized by considering their hydration. In solution, sucrose is unlikely to undergo thermal movement as the dry molecule. Rather, it will move as the hydrate, and the water available for freezing or equilibration with the vapor phase will be correspondingly reduced by an amount equal to the molal hydration,  $W_h$ . A new concentration,  $m'$ , may thus be defined:

$$m' = \frac{m}{(1 - W_h m)} \quad (1)$$

where  $W_h$  is the water of hydration, in kg H<sub>2</sub>O/mol solute. We may write

$$\phi = g' m' \quad (2)$$

where  $g'$  is the osmotic coefficient of the newly defined solution. To linearize the

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expression, we take the inverse of Eq. 1, and obtain

$$1/m = W_h + g'/\phi \quad (3)$$

An analogous relationship between inverse quantities is used for osmotic studies in cells and organelles. Recall that  $g$  approaches 1 as the solution approaches infinite *dilution*. Note especially that the intercepts Eq. 3 represent the approach to infinite *concentration*, rather than infinite dilution. *Thus, the intercepts cannot equal zero.*

Fig. 2 (Garlid, 2000) contains data for sucrose, PEG400, and BSA, plotted according to Eq. 3. Note that all three sets of data are linear with positive intercepts,  $W_h$ . More impressively, the slopes of the lines equal 1.0 within experimental error. This means that each of these solutes behaves as an *ideal* solute ( $g' = 1$ ) when a constant molar hydration is taken into account. Thus, hydrated sucrose is ideal to 2 molal.

The hydration values are instructive. In mol H<sub>2</sub>O/mol solute, they are 5 for sucrose, similar to the value obtained by Scatchard (1921), 22.7 for PEG400, and  $1.05 \times 10^4$  for BSA. The range is still large in g H<sub>2</sub>O/g solute: 0.28 for sucrose, 1 for PEG400 and 2.86 for BSA. These numbers indicate that sucrose is hydrated with one water per -OH group, whereas the large surface of BSA permits building up of water multilayers.

## 2. Osmotic equilibrium between normal aqueous phases—Theory

As previously stated, osmotic equilibrium is the state in which, in the absence of pressure gradients, water activity,  $a_w$ , is equal in the two phases. Thus, from its definition, the osmolality,  $\phi$ , is equal in the two phases, and

$$\Phi_i = \Phi_o \quad (4)$$

For internal osmolality, we will let

$$\Phi_i = g_i s_i \quad (5)$$

where  $s_i$  designates the ideal osmolality of the internal phase ( $s_i = \sum_j m_j$ ), and  $g$  is the osmotic coefficient. (It must be emphasized that  $g$  is a property of the *solution* and not of the individual solutes, as sometimes claimed.) Endogenous matrix solutes are not susceptible to direct measurement, but they may be written

$$S_i \equiv s_i W_i \quad (6)$$

where  $S_i$  is the amount (nosmol/mg protein) of osmotically active solute, and  $W_i$  is matrix water content. Combining Eqs. 5 and 6, we achieve an expression of osmotic equilibrium,

$$W_i = g_i S_i / \phi_o \quad (7)$$

As pointed out in the previous section, this proportionality cannot hold at infinite osmolality, where the influence of macromolecular hydration will be most extreme.

Thus, the correct equation for a perfect osmometer is

$$W_i = W_2 + g' S_1 / \phi_o \quad (8)$$

where  $W_2$  is the osmotic intercept (the water of hydration) and  $g'$  is the osmotic coefficient of the osmotically active phase. According to Eq. 8, water content should vary linearly with inverse osmolality, provided that there is no solute movement during the measurement ( $S_1$  is constant), and there should be a positive intercept.

### **3. Osmotic swelling in mitochondria due to changes in water activity**

Fig. 3 (Garlid, 2000) contains plots of matrix water content,  $W_i$ , vs. inverse osmolality. The data, which cover a 9-fold range of volume, are in complete agreement with the predictions of Eq. 8. Each curve is linear, and the slopes increase with increasing solute content. The curves have a common, non-zero intercept ( $W_2$ ), equal to 0.28 mg H<sub>2</sub>O/mg mitochondrial protein. Thus, when hydration is taken into account, the mitochondrion behaves as a perfect osmometer.

These results indicate that matrix water partitions into two aqueous phases, an osmotically active phase,  $W_1$ , and an osmotically inactive phase,  $W_2$ . The question whether  $W_2$  behaves as a solvent or represents “bound”, non-solvent water is addressed in the paper (Garlid, 2000).

### **4. Osmotically active matrix solutes and the osmotic slope**

How should matrix water content vary with changes in the osmotic strength of the medium. When we differentiate Eq 8 at constant  $S_1$ , we obtain

$$dW_i = g' S_1 d(1/\phi_o) \quad (9)$$

which tells us that the slope of the osmotic curve is  $g'S_1$ . This is a very important quantity, because  $S_1$  is the total osmotically active solute content (nmol/mg) of the matrix phase. (We have estimated  $g'$ , the osmotic coefficient of the "normal" phase, to be 0.94, and approximately constant, as is typical for a salt solution).

The major solutes of freshly isolated mitochondria are potassium salts of phosphate and organic anions. Experimentally, it is a simple matter to alter  $S_1$  by manipulating the  $Mg^{2+}$ -regulated  $K^+/H^+$  antiporter. We succeeded in preparing mitochondria which contained widely varying amounts of  $K^+$  salts and which did not lose these solutes during swelling.

Fig. 4 contains a plot of the osmotic slopes,  $S_1$ , versus matrix  $K^+$ , using the data in Fig. 3. The slope of the line is 1.52 nosmol/nmol  $K^+$ , and the intercept is 15 nosmol/mg. Uptake and loss of  $K^+$  salts constitutes the only significant change in matrix solutes in these experiments, and any net change in matrix  $K^+$  is accompanied by anions of average valence,  $z_a$ . The relationship between osmotic slope and  $K^+$  may be written

$$S_1 = g' \{s_1^o + (1 + 1/z_a) K^+\} \quad (10)$$

where  $s_1^o$  represents internal solutes not included in the second term in the equation.

The observed slope of 1.52 yields an estimated anion valency,  $z_a$ , of 1.62. This is in good agreement with expectations, since the labile anions are primarily phosphate and divalent anions under these conditions. This is an approximate analysis, because the distribution of  $K^+$  between phases 1 and 2 is unknown.

### **5. Osmotic swelling in mitochondria due to salt uptake**

As we know, mitochondria are continuously taking up and releasing  $K^+$  and other ions. The anion carriers are electroneutral and linked to the phosphate carrier, which effectively catalyzes equilibration of phosphoric acid. Inward  $K^+$  diffusion is matched 1:1 by  $H^+$  ions pumped out by the ETS. This perturbation of matrix pH will cause uptake of  $P_i$ , thereby restoring the proton to the matrix. What has changed? The matrix has

taken up  $K^+$  salts. This will invariably be associated with osmotically obligated water, according to Eq 8. Thus, at constant osmolality,

$$dW_i = (g'/\phi_o) dS_1 \quad (11)$$

In other words, an increase in solute content,  $dS$ , is *invariably* associated with an increase in matrix water.

This is extremely important physiologically. If  $K^+$  influx by diffusion were not compensated by the electroneutral  $K^+/H^+$  antiporter, mitochondrial water content would double every 5-10 minutes. In other words, they would burst.

### **6. Osmotically inactive matrix water and the osmotic intercept**

Three facts can be noted about the osmotic intercept. (i)  $W_2$  represents osmotically inactive *water*, as should be clear from the way the measurements were carried out. The suggestion frequently made that the osmotic intercept represents proteins and lipids is incorrect. (ii) A positive osmotic intercept is characteristic of all biological systems, as well as most inanimate systems, ranging from small nonelectrolytes to gels and coacervates. (iii) Osmotically inactive water is unaffected by changes in matrix solute concentrations.