2.7 Osmosis and Osmotic Pressure

Learning Objectives

- Define osmosis and osmotic pressure
- Write van't Hoff's limiting law for osmotic pressure dependent on concentration
- List the colligative properties of solutions and explain why vapor pressure depression perfectly predicts osmotic pressure
- Describe the osmotic coefficient for correction of nonideality in solutions
- Be able to calculate the predicted osmotic pressure for a solution
- Define hydraulic conductivity or hydraulic permeability
- Define the reflection coefficient
- Explain how the hydraulic conductivity depends on the microscopic parameters of the membrane
- Write an equation for volume flow across a microporous membrane in the presence of hydraulic and osmotic pressures
- Be able to calculate the hydraulic conductivity and reflection coefficient given appropriate data
- Describe the origin of the osmotic pressure for microporous membranes
- Describe how erythrocyte cell volume changes when placed in contact with solutions of varying [NaCl] or [glucose]
- Contrast the concepts of tonicity and osmolarity
- Describe the behavior of a perfect osmometer
- Explain why cells are not perfect osmometers
- Define RVD and RVI

OSMOSIS IS THE FLOW OF WATER DRIVEN BY SOLUTE CONCENTRATION DIFFERENCES

Probably no concept is more confusing to beginning students than osmosis and osmotic pressure, partly because it is defined backwards, as you will see. We begin with the experiments of Pfeffer in 1877.

Pfeffer made a precipitation membrane in the walls of unglazed porcelain cups by reacting copper salts with potassium ferricyanide. He used the precipitation membrane that resulted to separate a sucrose solution on the inside of the cup from water on the outside. He observed that water flowed from the outside to the inside. This is the primary observation of **osmosis**. **Osmosis refers to the movement of fluid across a membrane in response to differing concentrations of *solute* on the two sides of the membrane**. The word "osmosis" originates from the Greek, meaning "thrust" or "impulse."

Pfeffer also observed that the flow was proportional to the sucrose concentration inside the cup. When water was inside the cup, he observed that a pressure applied to the inside compartment would force water out of the cup, and this flow was proportional to the pressure. When sucrose was inside a closed cup, a pressure would develop inside the cup and this pressure was proportional to the sucrose concentration. He recognized this as an equilibrium state in which the outward filtration of water balanced the inward movement caused by osmosis. He *defined* the **osmotic pressure** as the **pressure necessary to stop osmotic flow across a barrier that is impermeable to the solute**. The osmotic pressure historically is given the symbol π (see Figure 2.7.1). This definition is the key: it defines the osmotic pressure as the pressure needed to stop osmotic flow rather than the pressure that drives osmotic flow. It is also defined only for a **semipermeable membrane**, one that is impermeable to solute but permeable to water.

THE VAN'T HOFF EQUATION RELATES OSMOTIC PRESSURE TO CONCENTRATION

Pfeffer's data (see Figure 2.7.2) showed that the osmotic pressure of solutions was linearly related to their concentration. In 1887, van't Hoff argued from Pfeffer's results, and from thought experiments considering gases in equilibrium with solutions, that the osmotic pressure should be given by

$$[2.7.1] \qquad \pi = RTC_s$$

where R is the gas constant ($0.082 \text{ L atm mol}^{-1} \text{ K}^{-1}$), T is the absolute temperature, and C is the molar concentration of solute in the inner compartment. Equation [2.7.1] is known as **van't Hoff's Law**. It gives the osmotic pressure due to the solute, s. In a mixture, the osmotic pressures due to each solute particle add up, much like Dalton's Law of Partial Pressures in gases. The result is that
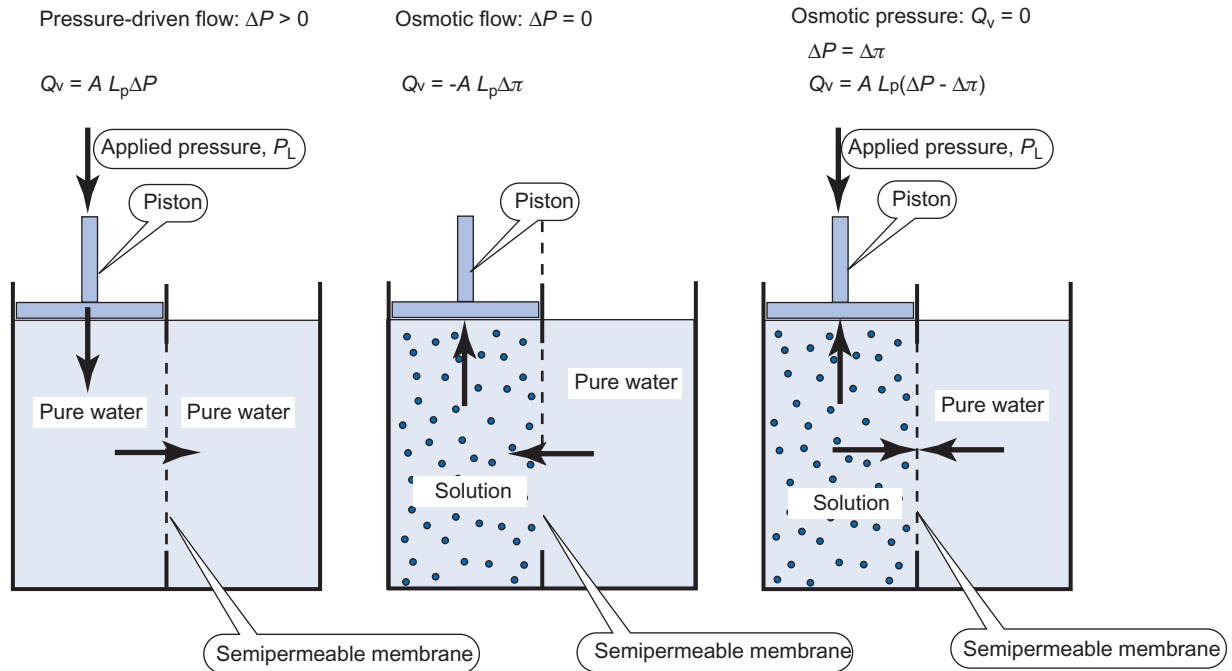
$$[2.7.2] \qquad \pi = RT \sum C_s$$

Pressure-driven flow: $\Delta P > 0$

$$Q_v = A\,L_p\Delta P$$

Applied pressure, P_L

Piston

Pure water | Pure water

Semipermeable membrane

Osmotic flow: $\Delta P = 0$

$$Q_v = -A\,L_p\Delta\pi$$

Piston

Pure water

Solution

Semipermeable membrane

Osmotic pressure: $Q_v = 0$

$$\Delta P = \Delta\pi$$

$$Q_v = A\,L_P(\Delta P - \Delta\pi)$$

Applied pressure, P_L

Piston

Pure water

Solution

Semipermeable membrane

FIGURE 2.7.1 Equivalence of hydrostatic and osmotic pressures in driving fluid flow across a membrane. Left panel: An ideal, semipermeable membrane is freely permeable to water but is impermeable to solute. When the membrane separates pure water on the right from pure water on the left, application of a pressure, P_L, to the left compartment forces water across the semipermeable membrane. The flow is linearly related to the pressure difference by the area of the membrane (A) and a proportionality constant, L_P, that is characteristic of the membrane. This constant is variously called the **hydraulic conductivity**, **hydraulic permeability**, or **filtration coefficient**. Positive Q_V is taken as flow to the right. Middle panel: The ideal, semipermeable membrane separates a solution on the left from pure water on the right, and water moves to the solution side by osmosis. The flow, Q_V, is linearly related to the difference in osmotic pressure, $\Delta\pi$, by the area of the membrane and the hydraulic conductivity, L_P. The flow causes expansion of the left compartment and movement of the piston, which is assumed here to be weightless. Right panel: Application of a pressure, P_L, to a solution so that $\Delta P = \Delta\pi$ results in no net flow across the membrane. The osmotic pressure of a solution is *defined* as the pressure necessary to stop fluid flow when an ideal semipermeable membrane separates pure water from the solution.
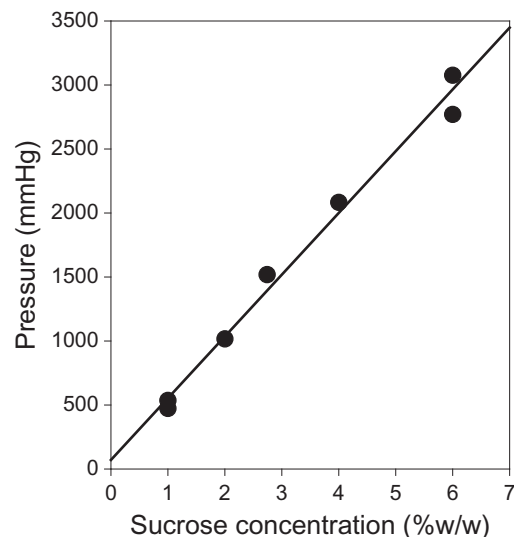

FIGURE 2.7.2 Plot of the data of Pfeffer (1877) for the osmotic pressure of sucrose solutions. A copper ferrocyanide precipitation membrane was formed in the walls of an unglazed porcelain cup. The membrane separated a sucrose solution in the inner chamber from water outside the cup. The inner chamber was then attached to a manometer and sealed. The linear relationship between the pressure measured with this device and the sucrose concentration was the experimental impetus for deriving van't Hoff's Law. *[Data from Pfeffer, W. Osmotische Untersuchungen Studien zur Zellmechanik, Leipzig [translated by G.R. Kepner and E.J. Tadelmann: Osmotic investigations: Studies on cell membranes, 1985, Van Nostrand Reinhold, New York].*

The concentration in van't Hoff's law, ΣC_s, refers to the *concentration of osmotically active solute particles in the solution*. Organic compounds such as glucose typically dissolve to form one particle for each molecule of solute, and for these compounds C_s is the same as the molar concentration. Strong salts, on the other hand, dissociate to form more than one particle for each mole of salt. NaCl, for example, dissociates in solution to form one Na^+ and one Cl^- ion. The concentration of osmotically active particles is twice the concentration of NaCl. Similarly, $CaCl_2$ dissociates nearly completely to form one Ca^{2+} ion and two Cl^- ions, and the total concentration of particles is 3 times the concentration of $CaCl_2$. The **osmolarity** of a solution equals ΣC_s and is expressed in osmoles per liter to indicate clearly that we are referring to the number of osmotically active solute particles, called **osmolytes**, rather than the concentration of the solute.

THERMODYNAMIC DERIVATION OF VAN'T HOFF'S LAW

We have learned that all spontaneous processes (those that occur naturally without any additional forces) are accompanied by a decrease in free energy. We have also learned that the free energy per mole is the chemical potential (see Chapter 1.7). In the case of a solution separated from pure water by a semipermeable membrane, water movement will occur when there is a difference in the chemical potential of *water* on the two sides of the membrane, such that water movement results in a decrease in the free energy. At equilibrium, when the pressure applied to the solution is equal to the osmotic pressure, the chemical potential of water is equal on both sides of the membrane, and no net movement of water occurs.

In the derivation that follows, we consider that a semipermeable membrane separates two compartments. On the right is pure water and on the left is some solution with concentration C_s. We know that there will be flow of water from the pure water to the solution side and that application of pressure, π, to the solution side will stop the flow. What is the relation between π and C_s? We can discover this by looking at the equilibrium condition when osmotic pressure balances hydrostatic pressure. At this point, the free energy change for water across the membrane is zero.

THE CHEMICAL POTENTIAL INCLUDES PRESSURE−VOLUME WORK

The equation we have used for chemical potential of a solute, the free energy per mole, is

[2.7.3] $\qquad \mu_s = \mu_s^0 + RT \ln C_s + z\Im\psi$

In this equation, $z\Im\psi$ represents the work done, per mole, in moving the material from the standard state to the condition in which the material is placed. In this case, it is the electrical work. There are other kinds of work such as pressure−volume work. The general equation for the chemical potential is

[2.7.4] $\qquad \mu_s = \mu_s^0 + RT \ln C_s + \text{work terms}$

where the work terms include all work (except concentration work, which is included explicitly in $RT \ln C_s$) necessary to bring the material from the standard state to its present state.

THE ACTIVITY CORRECTS THE CHEMICAL POTENTIAL FOR INTERACTIONS BETWEEN SOLUTE PARTICLES

It turns out that Eqn [2.7.4] is an approximation. In our derivation of the general Fick's Law, we did not consider some other kinds of interactions, such as solute molecules bumping into each other. The accurate equation is

[2.7.5] $\qquad \mu_s = \mu_s^0 + RT \ln a_s + \text{work terms}$

where a_s is the **activity** of the solute. In the case of osmosis, the work term is the pressure−volume work and there is no electrical work term. At equilibrium, where the pressure across the semipermeable membrane is the osmotic pressure, the chemical potential of water on the two sides of the semipermeable membrane must be equal (because the free energy change at equilibrium is zero). Therefore, we write the equality of chemical potential for water on the left and right sides as

$$\mu_w^0 + RT \ln a_{w,L} + \overline{V}_w P_L = \mu_w^0 + RT \ln a_{w,R} + \overline{V}_w P_R$$
[2.7.6]

where the subscripts L and R refer to the left and right sides of the semipermeable membrane, μ_w^0 is the chemical potential of liquid water in its standard state (pure water at 1 atm pressure), V_w is the volume of water per mole (the **partial molar volume**), P is the pressure, and a_w is the activity of water. For an **ideal solution**, the activity of water is its **mole fraction**:

[2.7.7] $\qquad a_w = X_w = \dfrac{n_w}{n_w + n_s}$

where X_w is the usual variable denoting the mole fraction of water, and n_w and n_s are the moles of water and solute, respectively, in any aliquot of the solution. Substituting in for a_w and canceling the μ_w^0 on both sides of Eqn [2.7.6], we come to

[2.7.8] $\qquad \overline{V}_w P_L - \overline{V}_w P_R = RT \ln X_{w,R} - RT \ln X_{w,L}$

Since the right-hand solution is pure water, $X_{w,R} = 1.0$ and $\ln X_{w,R} = 0$. Thus we have

[2.7.9] $\qquad \overline{V}_w(P_L - P_R) = -RT \ln X_{w,L}$

Now the mole fractions of solute and water in a solution must sum to 1.0. This is expressed as

[2.7.10]
$$X_{w,L} + X_{s,L} = 1.0$$
$$\ln X_{w,L} = \ln(1 - X_{s,L})$$

In dilute solutions, $X_{s,L} << 1.0$, so we may approximate $\ln(1 - X_{s,L}) \approx -X_{s,L}$. Substitution of this result into Eqn (2.7.9) gives

$$[2.7.11] \qquad (P_L - P_R) = \frac{RT}{\overline{V}_w} X_{s,L}$$

The left-hand side of Eqn [2.7.11] is just the osmotic pressure, π, which is equal to the extra pressure that must be applied to the left-hand side in order to establish equality of the chemical potential of water on the two sides of the membrane. From the definition of mole fraction, Eqn [2.7.11] becomes

$$[2.7.12] \qquad (P_L - P_R) = \pi = \frac{RT}{\overline{V}_w} \frac{n_s}{n_s + n_w}$$

For a dilute solution, $n_s << n_w$, so we approximate this result as

$$[2.7.13] \qquad \pi = \frac{RT}{\overline{V}_w} \frac{n_s}{n_w}$$

Again for a dilute solution, $n_w V_w \approx V$, the volume of the solution. Thus Eqn [2.7.13] gives

$$[2.7.14] \qquad \pi = RT \frac{n_s}{V}$$

or

$$[2.7.15] \qquad \pi = RTC_s$$

This last equation is the van't Hoff equation for the osmotic pressure. This thermodynamic derivation entails two assumptions: the solution is dilute enough to approach ideality and that the solution is incompressible so that the pressure–volume work is $V_w \Delta P$. It is important to recognize that the van't Hoff equation is not exact for physiological solutions. Rather, it is an approximation that is strictly true only for dilute ideal solutions.

OSMOTIC PRESSURE IS A PROPERTY OF SOLUTIONS RELATED TO OTHER *COLLIGATIVE PROPERTIES*

Osmotic pressure is closely related to some other properties of solutions, the **colligative properties**. These include the **freezing point depression**, the **boiling point elevation**, and the **vapor pressure depression**, all caused by dissolving solutes in a solution. The osmolarity is often determined from vapor pressure depression or freezing point depression, rather than from direct osmotic pressure measurements. The osmolarity is the concentration necessary to observe these phenomenon.

To see the connection between osmotic pressure and vapor pressure depression, consider Figure 2.7.3. A solution placed in a sealed container with a source of pure water will gain water because its vapor pressure is lower than that of the water. This situation is formally equivalent to osmosis, where the semipermeable membrane is the intervening air between the two surfaces.
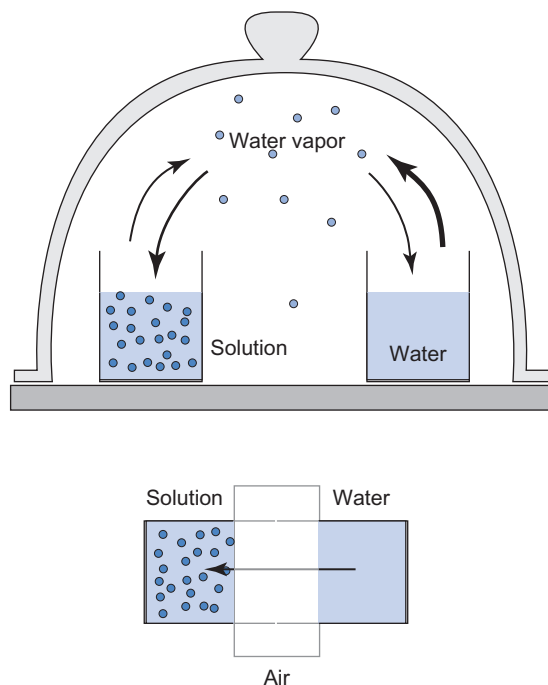

FIGURE 2.7.3 Equivalence of vapor pressure and osmotic pressure. Two beakers containing a solution or pure water are both placed in a single, sealed compartment. The air above the fluids contains air molecules plus water vapor. The partial pressure of water is its contribution to the total pressure, and it is proportional to the water concentration in the gas phase. The **vapor pressure** is *defined* as the **partial pressure of water in equilibrium with the liquid phase**. Water molecules will leave the liquid to moisten a dry gas. At equilibrium, molecules will evaporate from the liquid phase and condense from the gas phase at equal rates, so that a dynamic equilibrium is established. The vapor pressure of pure water is higher than the vapor pressure of the solution. This vapor pressure depression is one of the colligative properties of solutions. Because there is only one vapor pressure, it cannot simultaneously be in equilibrium with the water and with the solution. Thus water from the pure water beaker will continue to evaporate, because its vapor pressure is higher than that in the air, and water will continue to condense into the solution, because the partial pressure of water is higher than the vapor pressure of the solution.

Thus osmotic pressure and vapor pressure depression are perfect predictors of each other because essentially they are the same phenomenon.

THE OSMOTIC COEFFICIENT φ CORRECTS FOR THE ASSUMPTION OF DILUTE SOLUTION AND FOR NONIDEAL BEHAVIOR

As noted above, the van't Hoff equation makes two assumptions: the solution is dilute and it is ideal. The assumption of ideality enters when we equate the activity of water with its mole fraction. The assumption of dilute solutions allows us to identify $\ln(1 - X_s)$ with $-X_s$. We can correct for both assumptions by identifying

$$[2.7.16] \qquad \varphi = \frac{\pi_{observed}}{RTC_s}$$

Here φ is the **osmotic coefficient**. The osmotic coefficient can be less than or greater than 1.0.

THE RATIONAL OSMOTIC COEFFICIENT CORRECTS FOR THE ASSUMPTION OF IDEALITY

Equation [2.7.9] gives the osmotic pressure in terms of the mole fraction of water:

$$[2.7.17] \qquad \pi = -\frac{RT}{\overline{V}_w} \ln X_w$$

But the true equation for osmotic pressure is given by the manipulation of Eqn [2.7.6] as

$$[2.7.18] \qquad \pi = -\frac{RT}{\overline{V}_w} \ln a_w$$

We introduce the rational osmotic coefficient, *g*, to make these two equations give the same result:

$$[2.7.19] \qquad g \ln X_w = \ln a_w$$

The value of *g* can be calculated from Eqns [2.7.17] and [2.7.19] as

$$[2.7.20] \qquad g = \frac{\pi_{observed}}{-\dfrac{RT}{\overline{V}_w} \ln X_w}$$

Thus the rational osmotic coefficient corrects for the discrepancy between the osmotic pressure and the osmotic pressure calculated from the mole fraction of water. This assumes ideality, in which the activity is equal to the mole fraction, but not dilution. Thus the calculations of osmotic pressure based on Eqn [2.7.17] are better than those calculated using van't Hoff's Law because calculations based on Eqn [2.7.17] are valid even for solutions that are not dilute. However, Eqn [2.7.17] still requires the assumption of ideal solution behavior, or that the activity of water is equal to its mole fraction. Eqn [2.7.18] gives the osmotic pressure without assuming either a dilute solution or ideality. It can be calculated from vapor pressure measurements as

$$[2.7.21] \qquad \pi = -\frac{RT}{\overline{V}_w} \ln a_w = -\frac{RT}{\overline{V}_w} \ln \frac{p}{p^0}$$

Figure 2.7.4 shows the ratio of the observed osmotic pressure to that calculated by Eqn [2.7.15], Eqn [2.7.17], or Eqn [2.7.21]. These give an estimate of φ and *g*. The approximate values of φ at physiological concentrations for a variety of common solutes are given in Table 2.7.1. Units used in the calculation of osmotic pressure and appropriate values for *R* are given in Table 2.7.2.

EQUIVALENCE OF OSMOTIC AND HYDROSTATIC PRESSURES

As mentioned earlier, Pfeffer observed a linear relationship between the flow across the membrane and the pressure when water was on both sides of the membrane. This relationship can be described as

$$[2.7.22] \qquad \begin{aligned} Q_V &= A\, L_P(P_L - P_R) = AL_P\, \Delta P \\ J_V &= L_P(P_L - P_R) = L_P\, \Delta P \end{aligned}$$
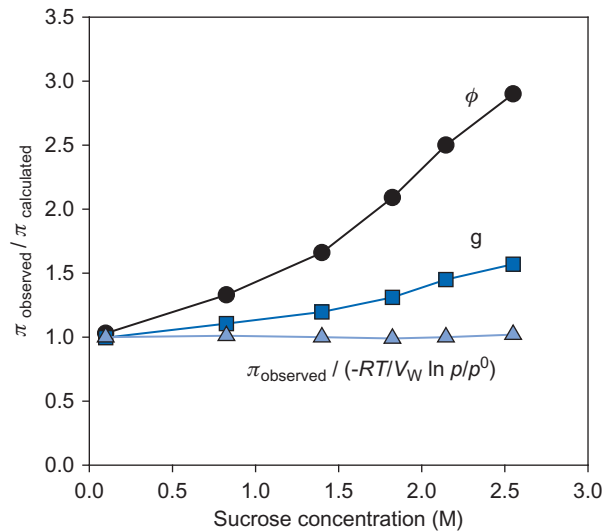

FIGURE 2.7.4 Osmotic coefficients as a function of sucrose concentration. The osmotic coefficient, φ, was calculated according to Eqn [2.7.16] by dividing the observed osmotic pressure by RTC (*circles*). The rational osmotic coefficient was obtained according to Eqn [2.7.20] by dividing the observed osmotic pressure by $-RT/\overline{V}_W \ln X_W$ (*squares*). The correlation of vapor pressure to osmotic pressure was tested by dividing the observed osmotic pressure by $-RT/\overline{V}_W \ln p/p^0$ (*triangles*). This figure shows that the van't Hoff equation is good for dilute solutions but fails at high solute concentrations, due to the failure of the assumptions that solutions are dilute and ideal. The osmotic coefficient corrects for these failures. The rational osmotic coefficient deviates significantly at higher sucrose solutions where solution behavior is further from ideal. The ratio of the observed osmotic pressure to that calculated from vapor pressure measurements is close to 1.0 over the entire concentration range. *Data from Glasstone, S.* Textbook of Physical Chemistry. *Princeton: Van Nostrand, 1946.*

TABLE 2.7.1 Approximate Values of the Osmotic Coefficient for Common Solutes Under Physiological Conditions

Solute	Number of Particles Formed upon Solution	Molecular Weight (g mol^{-1})	Osmotic Coefficient (φ)
NaCl	2	58.4	0.93
KCl	2	74.6	0.92
CaCl$_2$	3	111.0	0.85
Na$_2$SO$_4$	3	142.0	0.74
MgCl$_2$	3	95.2	0.89
MgSO$_4$	2	120.4	0.58
NaHCO$_3$	2	84.0	0.96
Alanine	1	89.1	1.00
Mannitol	1	182.2	1.00
Glucose	1	180.2	1.01
Sucrose	1	342.3	1.02

where Q_V is the flow in units of volume per unit time and J_V is the flux, or flow per unit area, in units of velocity. Here the positive flow is taken from left to right and pressure drives the flow. Thus if $P_L > P_R$, then

TABLE 2.7.2 Units for the Calculation of Osmotic Pressure

Pressure Units	1 atm Equivalent	Gas Constant (R)	Solute Osmolyte Concentration (ΣCs)[a]
atm	1	0.082 L atm mol^{-1} K^{-1}	mol L^{-1}
mm Hg	760	62.36 L mm Hg mol^{-1} K^{-1}	mol L^{-1}
Pa = N m^{-2}	1.013×10^5	8.314 N m mol^{-1} K^{-1}	mol m^{-3} = mol (1000 L)$^{-1}$
dyne cm^{-2}	1.013×10^6	8.314×10^7 dyne cm mol^{-1} K^{-1}	mol cm^{-3}

[a]Osmolarity (osmol L^{-1}) is defined as the concentration of osmotically active particles, osmolytes, in mol L^{-1}. Therefore, the units osmoles and moles cancel in the calculation of osmotic pressure.

Example 2.7.1 Calculate the Osmotic Pressure of 0.9% NaCl

0.9% NaCl means 0.9 g per 100 mL of solution. This is 9 g L^{-1}. We can convert this to molarity by dividing by the molecular weight, 58.4 g mol^{-1}: [NaCl] = 9 g L^{-1}/58.4 g mol^{-1} = 0.154 M.

The effective osmolarity of this solution is 2 osmol mol^{-1} × 0.154 M × 0.93 = 0.2866 osmol L^{-1}.

The osmotic pressure is calculated as $\pi = RT\,\varphi C =$ 0.082 L atm mol^{-1} K^{-1} × 310 K × 0.2866 mol L^{-1} = **7.29 atm**.

This is an enormous pressure on the physiological scale.

Q_V is positive and flow is to the right. If $P_L < P_R$, then $(P_L - P_R)$ is negative, Q_V is negative and flow is to the left. L_p is a coefficient characteristic of the membrane, variously called the **hydraulic conductivity**, **hydraulic permeability**, or **filtration coefficient**.

When Pfeffer added impermeant solutes to the inner chamber (left chamber), flow was observed into the solution in the absence of any macroscopic hydrostatic pressure differences. That is, when $\Delta P = 0$ there was a negative flow, and that flow was proportional to the osmotic pressure. Thus it appears that the solute caused a *reduction* in the pressure on the solution side because the flow is inward and additional pressure on the solution side, the osmotic pressure, is necessary to stop the osmotic flow. The osmotic pressure, which is characteristic of the solution, is equivalent to a reduction in the hydrostatic pressure. If solution is present on both sides of the membrane, we write

[2.7.23]
$$Q_V = A\,L_P[(P_L - \pi_L) - (P_R - \pi_R)]$$
$$Q_V = A\,L_P[(P_L - P_R) - (\pi_L - \pi_R)]$$
$$Q_V = A\,L_P(\Delta P - \Delta\pi)$$

THE REFLECTION COEFFICIENT CORRECTS VAN'T HOFF'S EQUATION FOR PERMEABLE SOLUTES

When membranes are partially permeable to the solute (leaky membranes), the measured osmotic pressure is less than that predicted by van't Hoff Law. This has led to the definition of another membrane parameter, σ, the **reflection coefficient**, which is *defined* as

[2.7.24]
$$\sigma = \frac{\pi_{\text{eff}}}{\varphi RTC}$$

where π_{eff} is the *effective* or observed osmotic pressure and φRTC is the theoretical osmotic pressure that would be observed if the membrane was perfectly semipermeable. The reflection coefficient is different for each pair of solute and membrane. Its range is $0 \leq \sigma \leq 1.0$. Rewriting Eqn [2.7.24], we have

[2.7.25]
$$\pi_{\text{eff}} = \sigma\varphi RTC = \sigma\pi$$

It is important to note that **the osmotic pressure is a characteristic of the solution**, and it is defined as the pressure necessary to stop osmotic flow when a solution is separated by an ideal, perfectly semipermeable membrane from pure water. Recall that a semipermeable membrane is defined as one that has zero permeability to solute. Every aqueous solution has an osmotic pressure—it is a concentration measure like molarity is—and it can be measured by any of the colligative properties. When a solution is placed in contact with a membrane that is not semipermeable, its osmotic pressure will be reduced by the membrane's reflection coefficient, which is characteristic not only of the membrane but also of the solute. As a consequence of this, flow across a real membrane that is not semipermeable will be altered. It is governed by the equation

[2.7.26]
$$Q_V = A\,L_P[(P_L - \Sigma_L\sigma_i\pi_i) - (P_R - \Sigma_R\sigma_i\pi_i)]$$
$$Q_V = A\,L_P(\Delta P - \Sigma\Delta\sigma_i\pi_i)$$

where the summation sign means that all osmotically active solutes in the solution contribute to the total osmotic pressure.

We now have three phenomenological coefficients that describe volume and solute flux across a membrane. These are summarized in Table 2.7.3. The question before us now is: what is the physical meaning of L_P and σ?

Example 2.7.2 Calculate the Net Driving Force and Flow Across a Dialysis Membrane.

A semipermeable dialysis membrane has an area of 90.5 cm² and $L_p = 6.46 \times 10^{-6}$ cm min⁻¹ mmHg⁻¹. Inside the dialysis membrane was a solution of 5% (w/v) sucrose at a pressure of 10 mmHg. Outside was a solution of 2% sucrose at a pressure of 50 mmHg. The entire apparatus was equilibrated to room temperature, 20°C. What is the net pressure across the membrane? What is the net flow across the membrane? Assume φ for sucrose is 1.0. The molecular weight of sucrose is 342 g mol⁻¹.

First, we calculate the osmotic pressures of the solutions. 5% sucrose means 5 g of sucrose per 100 mL of solution or 50 g L⁻¹. We can convert this to molarity by dividing by the molecular weight, 342 g mol⁻¹:

$$[\text{sucrose}]_{\text{in}} = 50 \text{ g L}^{-1}/342 \text{g mol}^{-1} = \textbf{0.146 M}.$$

The molar concentration of 2% sucrose is similarly calculated as

$$[\text{sucrose}]_{\text{out}} = 20 \text{ g L}^{-1}/342 \text{g mol}^{-1} = \textbf{0.058 M}$$

The osmotic pressure of the two solutions is calculated as

$$\pi_{\text{in}} = \varphi\, RT \times 0.146 \text{ M}$$
$$= 62.36 \text{ L mmHg mol}^{-1} \text{ K}^{-1} \times 293 \text{ K} \times 0.146 \text{ mol L}^{-1}$$
$$= \textbf{2668 mmHg}$$

$$\pi_{\text{out}} = \varphi\, RT \times 0.058 \text{ M}$$
$$= 62.36 \text{ L mmHg mol}^{-1} \text{ K}^{-1} \times 293 \text{ K} \times 0.058 \text{ mol L}^{-1}$$
$$= \textbf{1060 mmHg}$$

The net driving force is calculated as

$$(P_{\text{in}} - P_{\text{out}}) - (\pi_{\text{in}} - \pi_{\text{out}}) = (10 \text{ mmHg} - 50 \text{ mmHg})$$
$$- (2668 \text{ mmHg} - 1060 \text{ mmHg})$$
$$= -\textbf{1648 mmHg}$$

Positive pressure would force fluid outward; negative net pressure means flow is inward. Note that the systematic insertion of the pressure values is essential to obtaining the correct result.

The inward flow is given by

$$Q_V = A\, Lp(\Delta P - \Delta\pi)$$
$$= 90.5 \text{ cm}^2 \times 6.46 \times 10^{-6} \text{cm min}^{-1} \text{ mmHg}^{-1} \times -1648 \text{ mmHg}$$
$$= -\textbf{0.963 cm}^3 \textbf{ min}^{-1}.$$

TABLE 2.7.3 Phenomenological Membrane Coefficients

Coefficient	Parameter	Calculated as
p	Permeability	$(J_s/\Delta C)_{Jv=0}$
L_p	Hydraulic conductivity	$(J_v/\Delta P)_{\Delta\pi=0}$; $-(J_v/\sigma\Delta\pi)_{\Delta P=0}$
σ	Reflection coefficient	$-(J_v/L_p\Delta\pi)_{\Delta P=0}$

FIGURE 2.7.5 Schematic drawing of the hypothetical microporous membrane. We imagine that this membrane is a thin, flat sheet that is pierced by right cylindrical pores of radius a and depth δ, which is equivalent to the thickness of the membrane. There are $n = N/A$ pores per unit area of the membrane. The membrane separates two aqueous solutions with a solute concentration C_L on the left and C_R on the right. We further suppose that the left chamber is subject to the pressure P_L and the right side to the pressure P_R.

L_P FOR A MICROPOROUS MEMBRANE DEPENDS ON THE MICROSCOPIC CHARACTERISTICS OF THE MEMBRANE

Here we consider fluid flow across a microporous membrane such as that shown in Figure 2.7.5. We consider that the membrane itself is impermeant to water and solute, but both may go through tiny pores in the membrane. We consider three cases:

1. There is a pressure difference across the membrane but either there is water on both sides of the membrane or the solute particles are very small compared to the size of the pore. Pressure-driven flow will occur.
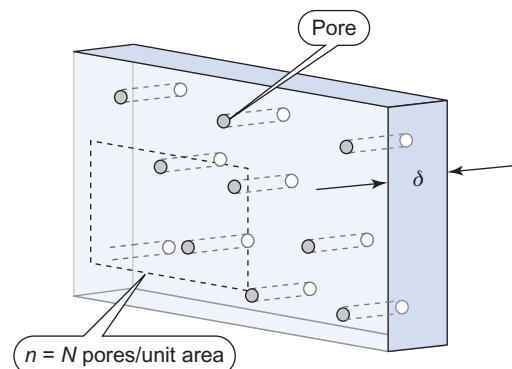2. There is no pressure difference across the membrane that separates water from solution, but the solutes on one side of the membrane are too large to fit through the pores. This will produce an osmotic pressure difference and an osmotic flow.
3. There is no pressure difference across the membrane that separates water from solution, but the solutes are small enough to fit through the pore, but not as easily as water. This will produce a smaller osmotic pressure and a smaller osmotic flow than in case 2.

CASE 1: THE SOLUTE IS VERY SMALL COMPARED TO THE PORE

The volume flow through the membrane is the number of pores times the volume flow per pore:

[2.7.27] $$Q_v = N \, q_v$$

where Q_V signifies the overall observed macroscopic flow, N is the number of pores, and q_v is the flow through a single pore. If we assume **laminar flow** (see Chapter 1.2), then the flow through each pore will be given by **Poiseuille's Law**, which gives the flow through a right cylindrical pipe of radius a and length δ as

[2.7.28] $$q_v = \frac{\pi a^4}{8\eta\delta}\Delta P$$

where η is the **viscosity** of the fluid. The use of Poiseuille's law in this situation requires the assumptions of laminar flow, Newtonian fluids, and a pore length that is long compared to the entrance length of the pore. The entrance length is the distance it takes for the fluid to establish a parabolic velocity profile within the pore. The observed macroscopic flux, J_v, is the flow per unit area of membrane per unit time. Substituting Poiseuille's law into Eqn [2.7.27] gives the volume flux as

[2.7.29] $$J_v = \frac{Nq_v}{A} = nq_v = \frac{n\pi a^4}{8\eta\delta}\Delta P$$

where $n = N/A$ is the density of pores in the membrane – the number of pores per unit area. Comparing this to Eqn [2.7.22], $J_V = L_P \, \Delta P$, we can identify

[2.7.30] $$L_p = \frac{n\pi a^4}{8\eta\delta}$$

According to this equation, the observed macroscopic flux is linearly related to the pressure difference by a coefficient which includes the thickness of the membrane, δ, the density of pores in the membrane, n, the radius of the pores, a, and the viscosity of the fluid, η. L_p has units of volume per unit time per unit area per unit pressure.

CASE 2: THE SOLUTE IS LARGER THAN THE PORE: THE MECHANISM OF OSMOSIS FOR MICROPOROUS MEMBRANES

If the solute particles are larger than the pore, then they cannot get through the membrane and the membrane is perfectly semipermeable. Pfeffer has already experimentally determined what happens in this case, and his experimental results permit several conclusions. First, the solute in the water causes the flow because there is no flow when pure water is on both sides of the membrane. Second, the pressure at equilibrium or the flow is directly proportional to the concentration of the solute, if the solutions are sufficiently dilute. Third, the solute causes a reduction in the pressure of the solution because the flow is inward and additional pressure on the solution side is necessary to stop the osmotic flow. Fourth, because the observed osmotic pressure obeys van't Hoff's Law only when the membrane is impermeable to solute, the osmotic pressure and the osmotic flow result from the interaction of the solute with the membrane. If the membrane is highly permeable to solute also, no osmosis and no osmotic pressure are observed. We should look to the interaction of the membrane with the solute to explain the mechanism of osmosis. The derivation for one mechanism of osmotic pressure flow and pressure generation is given in Appendix 2.7.A1. Briefly, this derivation recognizes that the total pressure in the bulk solution results from contributions of both solute and solvent molecules. However, solute molecules cannot enter the pores and so they cannot contribute to the pressure within the pore. Thus there is a pressure deficit on the solution side immediately upon entering the pore from that side. Since pressure results from collisions of molecules, resulting in momentum change in the molecules, there is a momentum deficit within the pores due to restricted entry of solute particles into the pores.

In Case 2, we have the situation where $P_L = P_R$, $C_L = C_L$, and $C_R = 0$ for a membrane which is impermeant to solute. From Eqn [2.7.A1.9], the pressure immediately within the pore, P_L', is given by

[2.7.31] $$P_L' = P_R - RTC_L$$

assuming the validity of van't Hoff's law. A plug of water extending the length of the pore is subjected to a pressure difference, which is given by

[2.7.32] $$P_L' - P_R = - RTC_L$$

because, in this case, $P_L = P_R$. The negative sign indicates the pressure is higher on the right than on the left. Thus fluid flow should occur from right to left according to Poiseuille's law:

[2.7.33] $$q_v = \frac{\pi a^4}{8\eta\delta}(-\pi)$$

where q_v is the flow per pore. In this equation, we see the absurd situation in which one symbol is used to signify two entirely different quantities. The π in the fraction is the geometric ratio, while the π in parenthesis signifies the osmotic pressure. The negative sign indicates flow to the left. The overall volume flux for the membrane is then given as

[2.7.34] $$J_v = N\frac{q_v}{A} = \frac{n\pi a^4}{8\eta\delta}(-\pi)$$

It is clear, then, that J_v in the presence of osmotic flow is given by

[2.7.35] $$J_v = - L_p \, \pi$$

where L_p is identical to that described earlier for pressure-driven flow (see Eqn [2.7.30]).
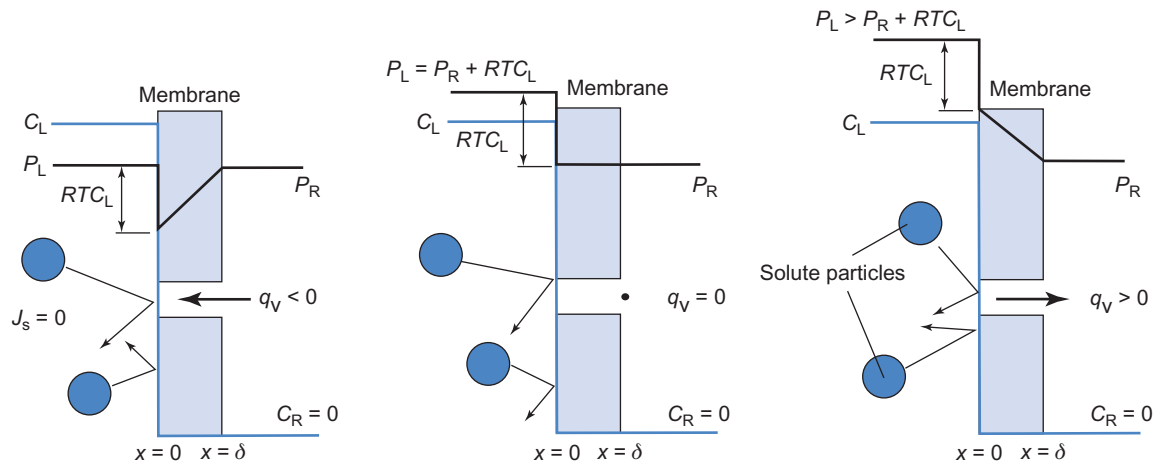
FIGURE 2.7.6 The direction of flow in the presence of hydrostatic and osmotic pressure differences across the membrane. Far left: The hydrostatic pressure is the same on both sides of the membrane but the left side contains a solution of concentration C_L. In the pore, there is a pressure gradient and flow is toward the solution. Middle: The pressure on the left was increased by $RTC_L = \pi$, the osmotic pressure. This is the equilibrium situation and no flow occurs. Right: Pressure on the left is more than RTC_L greater than pressure on the right, and flow is to the right.

It is instructive to consider the pressure profiles within the pore which are established due to the osmotic pressure. Poiseuille's Law is given as

$$[2.7.36] \qquad Q_v = \frac{\pi a^4}{8\eta}\left(\frac{\Delta P}{\Delta X}\right)$$

Since $\partial Q_v/\partial x = 0$ at steady state, it follows that $\Delta P/\Delta x$ must be a constant in x and in t: the pressure gradient is linear in x. This allows us to draw the schematic diagrams of pressure and concentration gradients during osmotic flow as shown in Figure 2.7.6.

CASE 3: THE REFLECTION COEFFICIENT RESULTS FROM PARTIALLY RESTRICTED ENTRY OF SOLUTES INTO THE PORES

When the membrane is freely permeable to the solute (Case 1), there is no osmosis and no osmotic pressure and hydrostatic pressure drives fluid flow. When the membrane is completely impermeable to the solute (Case 2), and there is no hydrostatic pressure, the osmotic pressure is ideally given by van't Hoff's Law and drives fluid flow. Here we consider Case 3, a membrane which distinguishes between solute and solvent but which is not completely impermeable to solute.

Here we consider pores that are large compared to the solvent particles but they *partially* exclude solute particles. The partial exclusion is due to the fact that the effective area of the pore is reduced compared to the area available to the solvent. When solute particles enter the pore, it reduces the effective osmotic pressure in direct proportion to the fraction of solute particles that can enter the pore. Consider that the pores have a radius a and that the solute particles are spherical with a radius a_s. If $a_s < a$, then at least some of the solute particles can get through the pore. Suppose that if a solute
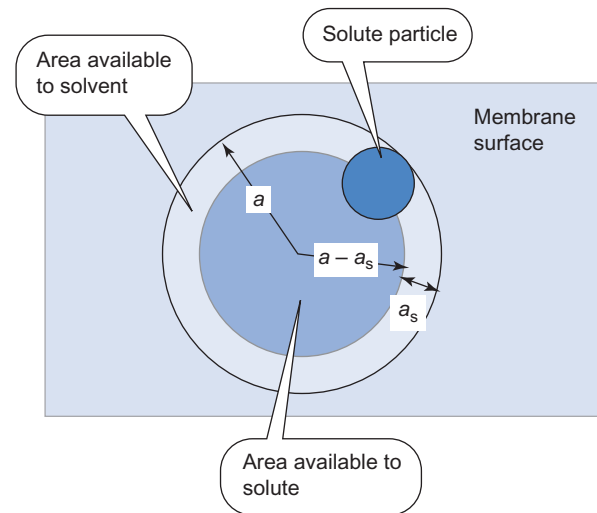

FIGURE 2.7.7 Relative areas of the pore available to solvent water and solute particles. The figure depicts a single pore, looking down along its axis normal or perpendicular to the surface of the membrane. The outer circle is the dimension of the pore that is available to the solvent water. The inner circle represents the dimensions of the pore available to the solute, which is less than that available to solvent.

particle hits the rim of the pore prior to entry, then it is *reflected* back into the bulk solution. The effective pore area will be reduced due to this reflection. The situation is depicted schematically in Figure 2.7.7, looking perpendicular to the membrane along the axis of the pore. The area of the pore which is accessible to solute is given by

$$[2.7.37] \qquad A_s = \pi(a-a_s)^2 = \pi a^2\left(1-\frac{a_s}{a}\right)^2$$

The relative area available to solute compared to that available to solvent is

$$[2.7.38] \qquad \frac{A_s}{A} = \frac{\pi a^2\left(1-\frac{a_s}{a}\right)^2}{\pi a^2} = \left(1-\frac{a_s}{a}\right)^2$$

The fraction of solute particles which are reflected by the pore is approximated by the ratio of the area in light blue in Figure 2.7.7 to the total area. This is identified with σ, the **reflection coefficient**:

$$[2.7.39] \quad \sigma = \frac{A - A_s}{A} = 1 - \frac{A_s}{A}$$

$$\sigma = 1 - \left(1 - \frac{a_s}{a}\right)^2$$

If $\xi = a_s/a$, then

$$[2.7.40] \qquad \sigma = 2\xi - \xi^2$$

We may expect the ratio of the concentration in the pore to the concentration in the bulk solution to be the same as the ratio of the pore area available to solute to the area available to solvent, A_s/A. This is given by Eqn [2.7.39] to be

$$[2.7.41] \qquad \frac{C'_L}{C_L} = \frac{A_s}{A} = 1 - \sigma$$

In the absence of solvent drag (when $J_v = 0$), the concentration profile may be drawn as shown in Figure 2.7.8.

In this model, some of the solute molecules can enter the pore and therefore they contribute to the pressure in the pore. We expect the pressure deficit within the pore to be due only to those molecules which are reflected. Thus the osmotic pressure should be $\sigma RT\Delta C = \sigma\Delta\pi$. The expression for laminar flow in the pore is

$$[2.7.42] \qquad J_v = \frac{n\pi a^4}{8\eta\delta}[\Delta P - \sigma\Delta\pi]$$

$$J_v = L_p[\Delta P - \sigma\Delta\pi]$$

In the above model, σ is viewed as being due to a hindered entry into the pore. There may be additional hindrance to solute flow through the pore due to interactions with the pore wall, but these are ignored here. According to this view, the reflection coefficient has
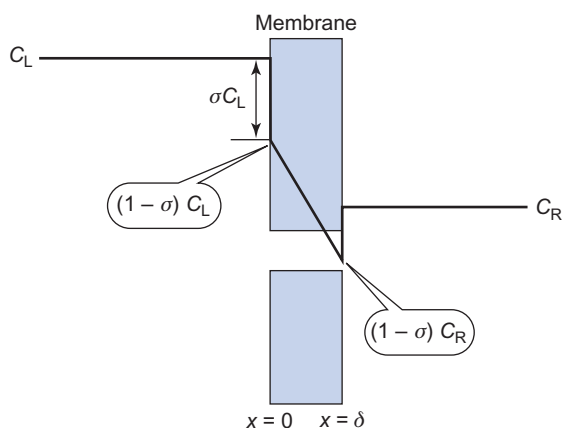
physical meaning only in the range $0 \le \sigma \le 1.0$. If $A_s = A$, then the pore is large enough so that the membrane does not discriminate between solute and solvent, and $\sigma = 0$ according to Eqn [2.7.39]. If $a_s \ge a$, then $A_s = 0$ and $\sigma = 1.0$.

SOLUTIONS MAY BE *HYPERTONIC* OR *HYPOTONIC*

When cells are placed in contact with a solution, they may either swell or shrink as shown in Figures 2.7.9 and 2.7.10. These observations introduce the idea of **tonicity**, which is operationally defined. If we place a cell in a solution and the cell swells, the solution is called hypotonic. If we place a cell in a solution and the cell shrinks, we call that solution hypertonic. If the cell neither swells nor shrinks, the solution is isotonic.

OSMOSIS, OSMOTIC PRESSURE, AND TONICITY ARE RELATED BUT DISTINCT CONCEPTS

Osmotic pressure is a theoretical concept. It is the pressure necessary to stop osmotic flow if a solution is separated from pure water by a semipermeable membrane. A semipermeable membrane is *defined* as a membrane that allows the passage of some molecules but not others. It is freely permeable to water but impermeable
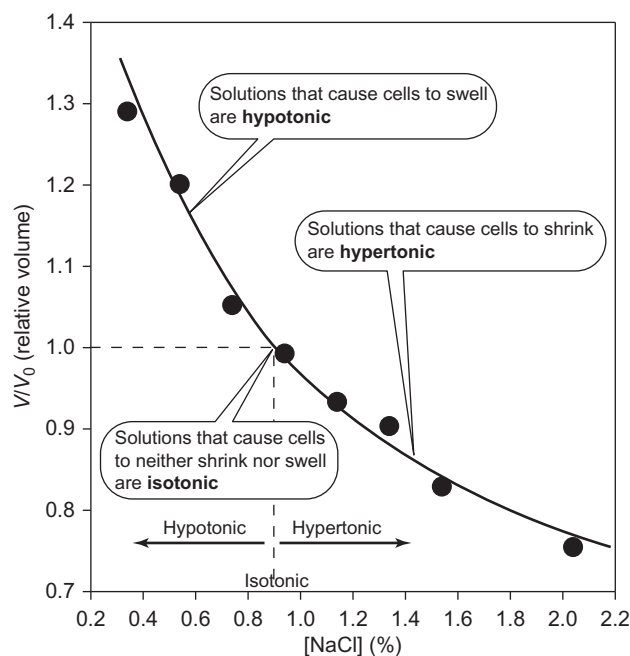
FIGURE 2.7.9 Swelling or shrinking of red blood cells in contact with different concentrations of NaCl. Whole blood was centrifuged to separate the red blood cells from the plasma. Then an aliquot of the plasma was removed and replaced with an equal volume of the indicated concentration of NaCl. When the plasma was replaced with NaCl solution less than about 0.9%, the cells' volume increased relative to that in normal plasma. These solutions are described as being **hypotonic**. When the [NaCl] that replaced plasma was greater than 0.9%, the red blood cells shrank. These solutions are called **hypertonic**. Replacement with 0.9% NaCl caused the cells to neither swell nor shrink: this solution is **isotonic**.

FIGURE 2.7.8 Concentration profile within a pore that partially excludes solute particles. This profile pertains only when solvent drag is zero. Solvent drag washes the concentration gradient in the direction of volume flow and alters J_s.

to solute. All solutions have an osmotic pressure. In the approximation of the ideal, dilute solution, the magnitude of the osmotic pressure is given by van't Hoff's Equation (see Eqn 2.7.7), where C is the molar concentration of osmotically active solutes. This C is the **osmolarity**.

Both osmolarity and osmotic pressure are properties of a solution, but tonicity is not. Tonicity refers to the direction of osmotic flow when a particular solution is placed in contact with a particular cell. Tonicity involves real membranes rather than an ideal, semipermeable membrane. The real membrane has a set of reflection coefficients, one for each solute on each side of the membrane. Whether shrinking or swelling occurs depends on the osmotic concentrations and compositions of the two solutions on the two sides of the cell membrane, and also on how these solutes interact with the real biological membrane.
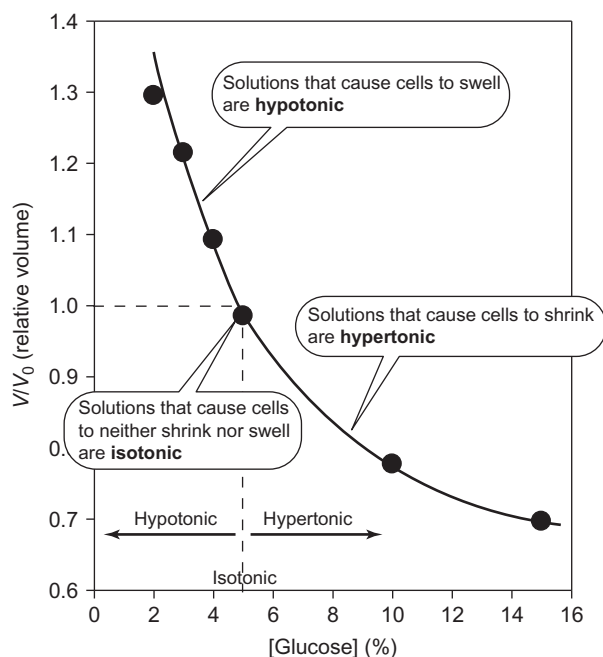

FIGURE 2.7.10 Swelling or shrinking of red blood cells in contact with different concentrations of glucose. Whole blood was centrifuged to separate the red blood cells from the plasma. Then an aliquot of the plasma was removed and replaced with an equal volume of the indicated concentration of glucose. When the plasma was replaced with solutions of glucose less than about 5%, the cells' volume increased relative to that in normal plasma. These solutions are described as being **hypotonic**. When the [glucose] that replaced plasma was greater than 5%, the red blood cells shrank. These solutions are called **hypertonic**. Replacement with 5% glucose caused the cells to neither swell nor shrink: this solution is **isotonic**.

CELLS BEHAVE LIKE OSMOMETERS

Consider a cell with a volume V_0 and a total concentration of osmotically active solutes, ΣC_i, resting in an isotonic medium. Now suppose that we rapidly replace the medium with another with a different osmotic pressure which we will symbolize as π. If the new medium is hypotonic, then by definition the cell will swell to a new volume, at which point the cell's cytoplasmic tonicity will match the medium's. Similarly, if the new medium is hypertonic, then the cell will shrink to a new volume that will match the tonicity of the medium. We assume here that the volume of the medium is so large that water movement into the cell or out of the cell does not appreciably affect the osmolarity of the medium. We further assume that water movement is fast compared to the movement of solutes or ions across the membrane, and that we can measure the cell's volume after water movement has occurred but before solute movement. What is the relationship between the medium osmotic pressure and the final cell volume, V_c?

In this situation, the total amount of osmotically active solutes in the cell is constant—the intracellular solutes do not move. The total intracellular osmolytes are equal to $V_0 \Sigma C_i$, where V_0 is the volume under isotonic conditions and C_i is the concentration of solute i in the cytoplasm in the isotonic condition. Now the total amount of osmotically active solutes at any time is

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Example 2.7.3 Isosmotic Solutions May Not Be Isotonic

A. Calculate the osmolarity and osmotic pressure of isotonic saline (NaCl):

Isotonic saline is 0.9% NaCl. This is 0.9 g NaCl per dL or 100 mL of solution = 0.9/0.1 L or 9.0 g L^{-1}.
The formula weight for NaCl is 58.44 g mol^{-1}. The *molarity* of NaCl is 9 g L^{-1}/58.44 g mol^{-1} = 0.154 M.
Since NaCl dissociates into two particles per mole, the osmolarity is 2 × 0.154 M = 0.308 OsM.
The osmotic coefficient of NaCl is $\varphi_{NaCl} = 0.93$, so that the measured osmolarity of this solution would be $\varphi \times C = 0.93 \times 0.308$ OsM = **0.286 OsM**. The osmotic pressure at 37°C is **7.27 atm**.

B. Calculate the osmolarity and osmotic pressure of 1.8% urea in water:

The formula weight for urea is 60.0 g mol^{-1} and $\varphi_{urea} = 0.95$.
Its osmolarity is $\varphi C = 0.95 \times 18$ g L^{-1}/60 g mol^{-1} = **0.285 OsM**. Its osmotic pressure is **7.24 atm**.

C. What happens when red blood cells are placed in 0.9% NaCl? 1.8% urea?

Red blood cells placed in contact with 0.9% NaCl neither shrink nor swell. The solution is isotonic. When placed in 1.8% urea, the cells swell so much that the cells lyse or break open. Although 0.9% NaCl and 1.8% urea are isoosmotic with red blood cell contents, 0.9% NaCl is isotonic and 1.8% urea is not isotonic.

distributed in the volume of the cell, V_c. So the osmolarity at any time is

[2.7.43] $$\frac{V_0 \sum C_i}{V_c}$$

When the osmotic pressure of the medium equilibrates with that of the cell, with the assumption of no solute movement (which is equivalent to assuming $\sigma_i = 1.0$), we get

[2.7.44] $$\pi = RT \frac{V_0 \sum C_i}{V_c}$$

This can be rewritten as

[2.7.45] $$\pi = \frac{V_0 \sum RTC_i}{V_c} = \frac{V_0}{V_c} \pi_{isotonic}$$

which we can rearrange to obtain

[2.7.46] $$V_c = V_0 \frac{\pi_{isotonic}}{\pi}$$

According to Eqn [2.7.46], a cell acting as a perfect osmometer would show a cell volume that was inversely proportional to the osmolarity of the external medium, with an intercept of zero. Typically the cell's volume under isotonic conditions, V_0, is the control for their volume under nonisotonic conditions. Thus we rewrite Eqn [2.7.46] as

[2.7.47] $$\frac{V_c}{V_0} = \frac{\pi_{isotonic}}{\pi}$$

Figure 2.7.11 shows the plot of the volume of cardiac cells exposed to various osmolar solutions, with volume normalized to the volume under isotonic conditions. According to Eqn [2.7.47], we expect the data to be linear with an intercept of zero. Actual data from these cardiomyocytes show that the response of relative volume (V_c/V_0) is linear within a considerable range,
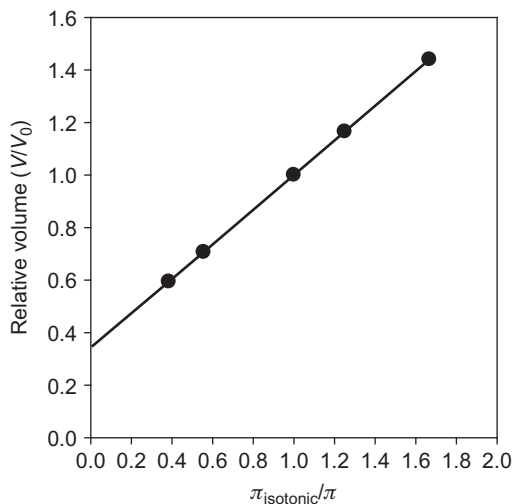

FIGURE 2.7.11 Relative volume of isolated cardiac cells exposed to test solutions of different osmolarities. Source: *Data from Drewnowska and Baumgarten, American Journal of Physiology **260**:C122–131, 1991.*

but the curve does not extrapolate to zero volume. These real cells are not perfect osmometers.

The response of real cells is described by the empirical equation

[2.7.48] $$\frac{V_c}{V_0} = \left(1 - \frac{V_b}{V_0}\right) \frac{\pi_{isotonic}}{\pi} + \frac{V_b}{V_0}$$

The intercept of this line on the volume axis is V_b/V_0, which in the case of cardiomyocytes shown in Figure 2.7.11 is 0.34. This is interpreted to mean that there is a fraction of the cell's volume that is **osmotically inactive**. This is partly to be expected. Not all of the volume of the cell is water, and it is the volume of water that dissolves osmotically active solutes and is responsive to changes in the medium osmolarity. Thus the sum of all of the volumes of large particles such as DNA, RNA ribosomes, and membranes contributes to an osmotically inactive volume. Although this is certainly part of the explanation for the osmotically inactive volume, the issue is by no means settled. Other components of the inactive volume might include the volume of a variety of macromolecular assemblies such as the cytoskeleton.

CELLS ACTIVELY REGULATE THEIR VOLUME THROUGH RVDs AND RVIs

Although the presence of a hypotonic or hypertonic solution initiates swelling or shrinking, respectively, often the volume change is not maintained. A cell that initially swells when placed in a hypotonic medium may eventually lose some of its acquired volume: it undergoes a **regulatory volume decrease or RVD**. The swelling of the cell activates compensatory mechanisms that cause transport of osmotically active solutes (osmolytes) out of the cell. Similarly, cell shrinking can activate an influx of osmolytes in some cells leading to a compensatory swelling that is called a **regulatory volume increase** or **RVI**. These RVDs and RVIs are accomplished by altering the cell's contents of osmolytes.

SUMMARY

Osmosis refers to the movement of fluid across a membrane in response to different concentrations of solutes on the two sides of the membrane. The movement of fluid is toward the more concentrated solution. Osmotic pressure is defined as the pressure that must be applied to the solution side to stop fluid movement when a semipermeable membrane separates a solution from pure water. Here, the semipermeable membrane is permeable to water but not to solute. The osmotic pressure for dilute ideal solutions obeys van't Hoff's Law:

$$\pi = RT\Sigma C_s$$

which can be derived on thermodynamic grounds. Because the solutions are not ideal, the equation is

refined by including an osmotic coefficient, φ_s, characteristic of each solute:

$$\pi_{observed} = RT\Sigma \; \varphi_s \; C_s$$

Defined in this way, the osmotic pressure is a pressure deficit caused by dissolving solutes. However, membranes that are somewhat permeable to the solute develop a transient osmotic pressure that is less than that predicted by van't Hoff's Law. Thus the actual pressure developed across a membrane that separates a solution from pure water depends on the interaction of the solute with the membrane. The correction for partially permeable membranes requires the reflection coefficient, σ:

$$\pi_{observed} = RT\Sigma \; \sigma_s \; \varphi_s \; C_s$$

The magnitude of the pressure tells us nothing of the flow. Osmotic pressure and hydrostatic pressure add to drive fluid flow across the membrane, with a proportionality constant, L_p. The phenomenological equation describing fluid flow is

$$Q_v = A\,L_p(\Delta P - \sigma\Delta\pi)$$

Osmosis and osmotic pressure is a thermodynamic concept which exists independently of mechanism. In microporous membranes, osmosis is caused by a momentum deficit within the pores due to the reflection of solute molecules by the membrane. This reduces the pressure on the solution side of the pore by π for a semipermeable membrane.

Thus there are three characteristic parameters for describing passive material transfer across membranes: the permeability, p, the hydraulic conductivity, L_p, and the reflection coefficient, σ.

Osmolarity is a kind of concentration measure, distinct from molarity. It is related to other colligative properties of solutions including freezing point depression, vapor pressure depression, and boiling point elevation. Tonicity is a related concept but involves a real, biological membrane that may not be semipermeable. Tonicity makes reference to a particular cell and its membrane. Solutions may be isoosmotic but not isotonic.

Cells respond to swelling or shrinking according to the empirical relation:

$$V/V_0 = (1 - V_b/V_0)\,\pi_{isotonic}/\pi + V_b/V_0$$

where V_0 is the volume of the cell under isotonic conditions. V_b is interpreted as the osmotically inactive volume. If $V_b = 0$, the cell would behave like an ideal osmometer.

REVIEW QUESTIONS

1. In which direction does osmotic flow occur? Why?
2. What equation gives the magnitude of the osmotic pressure? What are the limitations of this equation? How would you correct for its errors?
3. What is the reflection coefficient? How does it relate to permeability?
4. The equations in this chapter were derived for a microporous membrane. Would they still hold for a lipid dissolution model of a membrane?
5. How does tonicity differ from osmolarity? Define hypotonic, hypertonic, and isotonic.
6. What is the relationship between volume and osmotic pressure in a perfect osmometer?
7. What is meant by "osmotically inactive" volume?
8. How do cells regulate their volume?

APPENDIX 2.7.A1 MECHANISM OF OSMOSIS: FILTRATION VERSUS DIFFUSION DOWN A CONCENTRATION GRADIENT

PHYSICAL ORIGIN OF THE OSMOTIC PRESSURE ACROSS A MICROPOROUS MEMBRANE

Consider what happens in the vicinity of a single pore, as shown in Figure 2.7.A1.1. Since the pore excludes solute particles, the average concentration must decrease from C_L in the bulk solution to zero in moving along the axis of the pore. Thus there is a concentration gradient near the opening of the pore. By Fick's First Law of Diffusion, we should expect a net diffusion of solute particles into the pore. However, the particles hit the rim of the pore and are reflected back into the bulk solution. On the average, these particles experience a force in the $-x$ direction. The magnitude of this average force can be obtained from the generalized Fick's equation (see Eqn [1.6.42]):

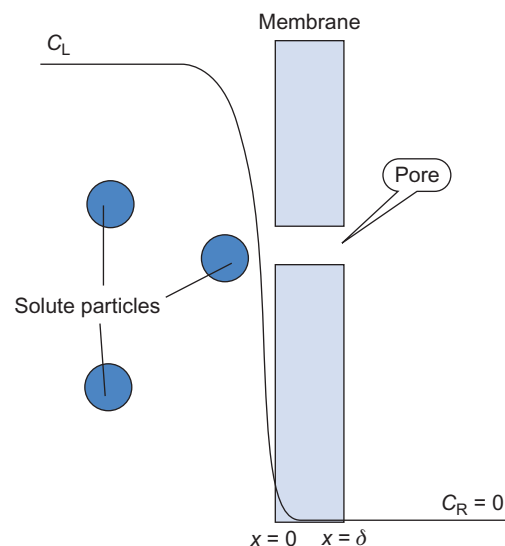[2.7.A1.1] $$j_s = -D\frac{\partial C(x)}{\partial x} + \frac{D}{RT}F\,C(x)$$

FIGURE 2.7.A1.1 Concentration gradient for solute particles near a pore. In this case, the solute particles are large compared to the pore and cannot penetrate into the membrane.

We write R in place of k and F in place of f in Eqn [2.7.A1.1] because we are speaking of the force per mole rather than the force per molecule, as was done in Eqn [1.6.42].

Since $j_s = 0$ for an impermeant membrane, Eqn [2.7.A1.1] becomes

[2.7.A1.2] $$F C(x) = RT \frac{\partial C(x)}{\partial x}$$

The force F is an external force that acts on the solute particles. The total force acting on the solute particles is just $Fn(x)$, where n is the number of moles of particles. Dividing by the volume, V, we have $Fn(x)/V = FC(x)$, which is the average force per unit volume. This is the force that the membrane is exerting on the solute bodies, per unit volume, located in the volume of fluid directly in front of the pore. The consequence of this is that there will be a pressure drop at the pore entrance. To see this, we will analyze the forces acting on an element of fluid located directly in front of a pore, as shown in Figure 2.7.A1.2.

Here we consider the forces acting on an element of fluid with an area A from a point x well within the bulk solution to a point $x + \Delta x$ within the pore near its surface. We consider that the volume element is in mechanical equilibrium. The forces acting on this volume are the forces acting on the solute particles and the forces acting on the surfaces in contact with adjacent fluid. The sum of these forces must be zero to meet the condition of mechanical equilibrium. We identify these forces with the "body" forces, F_B, and "contact" forces, F_C. Thus we write:

[2.7.A1.3] $$0 = F_B + F_C$$

for the condition of mechanical equilibrium. The net contact force is the balance of the pressure acting on the surface area to the right and left of the volume element:

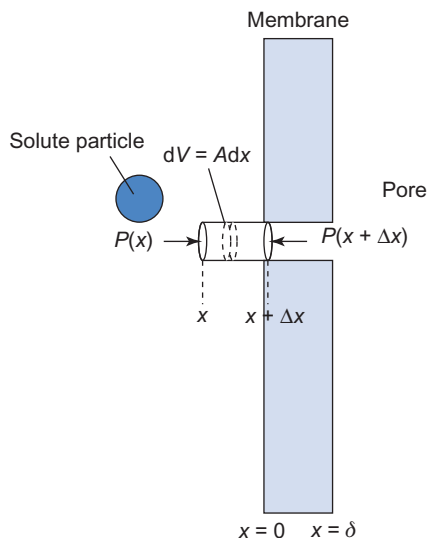[2.7.A1.4] $$F_C = A P(x) - A P(x + \Delta x)$$

FIGURE 2.7.A1.2 Forces acting on the plug of fluid immediately in front of a pore in a porous membrane.

The total body force is the body force per unit volume integrated over the volume.

[2.7.A1.5] $$F_B = \int_x^{x+\Delta x} F C(x) dV$$

Inserting $dV = A dx$ and $FC(x) = RT \ \partial C(x)/\partial x$ from Eqn [2.7.A1.2], we obtain

[2.7.A1.6]
$$F_B = ART \int_x^{x+\Delta x} \frac{\partial C(x)}{\partial x} dx$$
$$= ART[C(x + \Delta x) - C(x)]$$

Since $C(x + \Delta x) = 0$ because solute particles are not in the pore, Eqn [2.7.A1.6] becomes

[2.7.A1.7] $$F_B = -ART \ C(x)$$

The negative sign in Eqn [2.7.A1.7] indicates that F_B is directed to the left. Inserting Eqns [2.7.A1.4] and [2.7.A1.7] into Eqn [2.7.A1.3], we obtain

[2.7.A1.8] $$A P(x) - A P(x + \Delta x) = ART \ C(x)$$

or

[2.7.A1.9] $$P(x) - P(x + \Delta x) = RTC_L$$

This equation says that the net pressure experienced by the volume of fluid immediately adjacent to the pore decreases as one moves from the left compartment into the pore, and that the drop in pressure is RTC_L. This analysis suggests that the osmotic pressure develops as a consequence of the interactions between the solute particles and the membrane. The solute particles contribute momentum in the solution. When the particles collide with the membrane, that momentum is transferred to the membrane and not to the fluid within the pore. As a consequence, there is a momentum deficit within the pore (compared to the bulk solution). Since the pressure is the average momentum change per unit area experienced by particles colliding within the fluid, this momentum deficit shows up as a pressure deficit. The resulting pressure difference produces a flow that is given as

[2.7.A1.9] $$J_v = \frac{n\pi a^4}{8\eta\delta} \Delta\pi$$

where we identify

[2.7.A1.10] $$L_p = \frac{n\pi a^4}{8\eta\delta}$$

DIFFUSIONAL PERMEABILITY OF MICROPOROUS MEMBRANES

In the absence of a hydrostatic or osmotic pressure gradient, water will diffuse across a microporous membrane. If we assume the membrane is impermeable

except at the pores, the diffusion through the pores will obey Fick's Laws of diffusion:

[2.7.A1.11]
$$j_w = -D\frac{\partial C}{\partial x}$$
$$\frac{\partial C}{\partial t} = D\frac{\partial C^2}{\partial x^2}$$

where j_W is the flux through a single pore. At steady state, the flux does not change with time, or with distance, and so the gradient is linear in x and we can write

[2.7.A1.12]
$$j_w = \frac{\pi a^2 D_w}{\delta}\Delta C_w$$

where j_w is the flux of water through the pore, a is the radius of the pore, D_W is the diffusion coefficient of water in the pore, δ is the length of the pore (equal to the thickness of the membrane), and ΔC_W is the concentration difference of water. The overall macroscopic flux for a microporous membrane is given as

[2.7.A1.13]
$$J_w = \frac{n\,\pi a^2 D_w}{\delta}\Delta C_w$$

where n is the density of the pores, $n = N/A$, the number of pores per unit area of the membrane. This is analogous to the passive solute flux discussed in Chapter 2.5. We can similarly define a diffusional permeability for water from the relation:

[2.7.A1.14]
$$J_w = P_d\,\Delta C_w$$

and we can identify a **diffusional permeability coefficient** for water from Eqns [2.7.A1.13] and [2.7.A1.14] as

[2.7.A1.15]
$$P_d = \frac{n\,\pi a^2 D_w}{\delta}$$

FILTRATION PERMEABILITY IN THE PRESENCE OF A PRESSURE DIFFERENCE IN A MICROPOROUS MEMBRANE

In Chapter 1.6, we found that diffusional flux can be altered by the application of an additional force. In particular, a flux of solute obeyed the relation:

[2.7.A1.16]
$$J_s = -\frac{D}{RT}C\,F$$

where J_S is the flux of solute, D is its diffusion coefficient, R is the gas constant, T is the temperature (K), C is the concentration, and F is the total force on the particle per mole. We went on to define the electrochemical potential so that

[2.7.A1.17]
$$F = -\frac{d\mu}{dx}$$

These equations have general validity and are also true for water. We can write for the flux in the pore that

[2.7.A1.18]
$$j_w = \frac{D_w}{RT}C\frac{d\mu_w}{dx}$$

From the thermodynamic derivation of van't Hoff's Law, we write for the chemical potential of water

[2.7.A1.19]
$$\mu_w = \mu_w^0 + RT\ln a_w + \overline{V}_w P$$

insertion of this into Eqn [2.7.A1.18] and at steady-state flow, we obtain for the macroscopic membrane

[2.7.A1.20]
$$J_w = \frac{n\pi^2 D_w}{RT\delta}C_w\overline{V}_w\Delta P$$

V_w is the partial molar volume (in volume per mole, $L\,mol^{-1}$), and C_W is the water concentration, in moles L^{-1}, and these cancel: $V_W C_W = 1.0$. The flux given here is in mole per unit area per unit time. To convert to J_V we multiply by V_W:

[2.7.A1.21]
$$J_V = \frac{\overline{V}_w n\pi a^2 D_w}{RT\delta}\Delta P$$

This describes pressure-driven flow across the membrane. We recognize that the same terms for P_d are present in this equation. We recognize Eqn [2.7.A1.21] as $J_V = L_P\,\Delta P$ and identify L_P as

[2.7.A1.22]
$$L_P = \frac{\overline{V}_w n\pi a^2 D_w}{RT\delta} = \frac{\overline{V}_w P_f}{RT}$$

where P_f is the **filtration permeability for water**. It has the same expression for P_d given in Eqn [2.7.A1.15] but we obtain it experimentally from L_P as

[2.7.A1.23]
$$P_f = L_P\frac{RT}{\overline{V}_w} = \frac{n\,\pi a^2 D_w}{\delta}$$

Thus comparing Eqn [2.7.A1.15] to Eqn [2.7.A1.23], P_d and P_f should be the same if the mechanism of osmosis is by diffusion.

PRESSURE- AND OSMOSIS-DRIVEN FLOW ACROSS A LIPID BILAYER BY DISSOLUTION−DIFFUSION

Here we consider a membrane that presents all of its area to the solution phase, and water crosses by dissolving in the lipid of the membrane on one side of the membrane, diffusing across the membrane essentially like a vapor, and then coming out of lipid solution back into the aqueous phase on the other side. We imagine that dissolution is rapid (the solution phase and membrane phase are in equilibrium) and that diffusion is comparatively slow. Equilibrium of water in the solution phase with water in the membrane phase is described by equating the chemical potential of water in the two phases:

$$\mu_{w(solution)}^0 + RT\ln X_{w(solution)} + \overline{V}_{w(solution)}P$$
$$= \mu_{w(membrane)}^0 + RT\ln X_{w(membrane)} + \overline{V}_{w(membrane)}P$$

[2.7.A1.24]

where $X_{w(solution)}$ and $X_{w(membrane)}$ are the mole fractions of water in the solution in equilibrium with the

membrane and in the membrane phase, respectively. The partition coefficient is defined as

$$[2.7.A1.25] \qquad K_w = \frac{X_{w(membrane)}}{X_{w(solution)}}$$

Consider the case where only osmotic pressure drives water flow and the hydrostatic pressure difference across the membrane is zero. Since water generally partitions poorly into hydrocarbon solvents, we may assume that the mole fraction of water in the membrane phase is low. Thus the water concentration is dilute, and we may replace the mole fraction of water with its concentration:

$$[2.7.A1.26] \qquad C_{W(membrane)} \approx \frac{X_{w(membrane)}}{\overline{V}_{lipid}}$$

If equilibrium is rapid, we can combine Eqn [2.7.A1.25] and Eqn [2.7.A1.26] to get

$$[2.7.A1.27] \qquad C_{w(membrane)} \approx K_w \frac{X_{w(solution)}}{\overline{V}_{lipid}}$$

For dilute solutions, $X_{W(solution)}$ can be replaced by $1 - \overline{V}_w C_S$ where C_S is the solute concentration:

$$[2.7.A1.28] \qquad C_{w(membrane)} \approx K_w \frac{1 - \overline{V}_w C_S}{\overline{V}_{lipid}}$$

The concentration of water immediately inside the left-hand side of the membrane is given by Eqn [2.7.A1.28] where C_S is the concentration of solute in the left compartment. A similar expression pertains to the water concentration immediately inside on the right side. The net diffusive flux of water across the membrane is given as

$$[2.7.A1.29] \qquad J_w = -D_w^m \frac{C_{w,L} - C_{w,R}}{0 - \delta}$$

where D_w^m is the diffusion coefficient of water in the membrane phase. Substituting in for the concentrations from Eqn [2.7.A1.28], we get

$$J_w = -\frac{D_w^m K_w \overline{V}_w (C_{S,L} - C_{S,R})}{\overline{V}_{lipid}\, \delta} = -\frac{D_w^m K_w \overline{V}_w \Delta C_S}{\overline{V}_{lipid}\, \delta}$$
$$[2.7.A1.30]$$

The flux given here is in mole per unit area per unit time. To convert to J_V, we multiply by V_w:

$$J_V = \overline{V}_w J_w = -\frac{D_w^m K_w \overline{V}_w^2 \Delta C_S}{\overline{V}_{lipid}\, \delta} = -\frac{D_w^m K_w \overline{V}_w^2}{\overline{V}_{lipid}\, RT\, \delta} RT \Delta C_S$$
$$[2.7.A1.31]$$

The last term on the far right is the osmotic pressure difference, $\Delta\pi$. Equation [2.7.A1.31] relates the volume flux to the osmotic pressure when the mechanism of water flow is dissolution and diffusion. We recognize it as a form of the phenomenological equation, $J_V = -L_P \Delta\pi$ and therefore we identify L_P as

$$[2.7.A1.32] \qquad L_P = \frac{D_w^m K_w \overline{V}_w^2}{\overline{V}_{lipid}\, RT\, \delta}$$

In Eqn [2.7.A1.23], we described P_f, the filtration water permeability, as

$$[2.7.A1.23] \qquad P_f = L_P \frac{RT}{\overline{V}_w}$$

Applying this result to Eqn [2.7.A1.32], we obtain

$$[2.7.A1.33] \qquad P_f = \frac{D_w^m K_w \overline{V}_w}{\overline{V}_{lipid}\, \delta}$$

This equation was derived for the situation in which there was an osmotic gradient ($\Delta C_S > 0$) in the absence of a hydrostatic pressure gradient ($\Delta P = 0$). The expression for the situation where $\Delta C_S = 0$ and $\Delta P > 0$ can be obtained by returning to Eqn [2.7.24] and setting the mole fractions of water to 1.0 while the pressures differ. The result is that exactly the same L_P is derived for pressure-driven flow as for osmotic flow when the mechanism is by rapid dissolution of water followed by slow diffusion of water through the membrane phase.

DIFFUSIONAL PERMEABILITY BY THE DISSOLUTION–DIFFUSION MECHANISM: P_D

The permeability of lipid membranes to a diffusional water flux is expressed by the equation:

$$[2.7.A1.34] \qquad J_w = P_d \Delta C_w$$

where P_d is the diffusional permeability and ΔC_w is the difference in water concentration across the membrane. The overall permeation of water takes three steps: dissolution into the membrane phase at the left interface, diffusion across the membrane phase, and reversal of the dissolution at the right interface. If we assume, as we did in the derivation of P_f, that the rate-limiting step is diffusion through the membrane phase, then we may rewrite Eqn [2.7.A1.34] as

$$[2.7.A1.35] \qquad J_w = D_w^m \frac{\Delta C_{w(membrane)}}{\delta}$$

Substituting in for $\Delta C_{w(membrane)}$ from Eqn [2.7.A1.27], this is

$$[2.7.A1.36] \qquad J_w = D_w^m \frac{K_w \Delta X_{w(solution)}}{\overline{V}_{lipid}\, \delta}$$

since $\Delta X_w = V_w\, \Delta C_w$, this becomes

$$[2.7.A1.37] \qquad J_w = D_w^m \frac{K_w \overline{V}_w}{\overline{V}_{lipid}\, \delta} \Delta C_w$$

and we can identify P_d by comparing Eqns [2.7.A1.37] and [2.7.A1.34] as

$$[2.7.A1.38] \qquad P_d = \frac{D_w^m K_w \overline{V}_w}{\overline{V}_{lipid}\, \delta}$$

Comparing the results for P_f (see Eqn [2.7.A1.33]) and for P_d (see Eqn [2.7.A1.38]), the dissolution–diffusion mechanism of water transport indicates that $P_f/P_d = 1.0$.

If we measure the diffusional permeability of a lipid membrane and L_P, and calculate P_f from the L_P according to Eqn [2.7.A1.23], we should expect them to be equal provided that the mechanism is dissolution and diffusion. Our earlier results with microporous membranes also concluded that $P_f/P_d = 1.0$ if the mechanism of transport was by diffusion.

EXPERIMENTS CONFIRM $P_F/P_D = 1.0$ FOR LIPID BILAYERS BUT $P_F/P_D >> 1.0$ FOR MOST BIOLOGICAL MEMBRANES

The measurement of P_f (from L_P) and P_d in planar lipid bilayers confirm that they are the same, indicating that water transfer across pure lipid bilayer membranes is by diffusion. However, in most biological membranes, P_f/P_d is much greater than 1.0, suggesting that the mechanism is not diffusional, but hydrodynamic. The erythrocyte membrane was used in many of these studies, and it was shown that compounds that interact with protein sulfhydryl groups markedly reduced P_f while leaving P_d relatively unchanged, and causing the ratio of P_f/P_d to become 1.0. These early studies pointed to the existence of proteinaceous pores in the erythrocyte membrane, and subsequently these were identified as AQP1, the first in the family of aquaporins. Peter Agre earned the Nobel Prize in 2003 for his discovery of the aquaporins.

AQUAPORINS ACCOUNT FOR MOST WATER TRANSPORT IN CELLS

Aquaporins are a family of proteins, all about 28 kDa, that are found in a variety of tissues that have high water transport rates such as the intestines and kidneys, salivary glands, pancreas, and more. There are 13 human varieties, labeled AQP0, AQP1−12. The proteins all have six transmembrane domains and associate to form a functional tetramer, although each part has its own water channel. Although water can permeate through lipid membranes, this pathway is much slower than the AQP-mediated pathway. Thus real biological membranes are a mosaic of lipid pathways for diffusional transport of water and pores for pressure-driven transport of water.