

Osmosis In Plant Cells

There are no well-documented cases of active transport of water into plant cells. Instead, water appears only to cross the differentially-permeable cell membrane by a process analogous to diffusion (the passive movement of chemicals from an area of greater concentration to an area of less concentration). This movement of water is called OSMOSIS.

Since osmosis does not require expenditure of energy, it must be an energetically "downhill" process. The measure of the energy involved in osmosis is called WATER POTENTIAL (Ψ) expressed in a variety of units (atm, bar, MPa). In this exercise we will use MPa as the common unit. Since the water must lose energy as it moves by osmosis, water must move from an area of GREATER potential to an area of LESS water potential:

- If the water potential is greater inside the cell than outside the cell, then there will be a net movement of water OUT of the cell.
- If the water potential is greater outside the cell than inside the cell, then osmosis will be a spontaneous net movement of water INTO the cell.
- If the water potential on each side of a cell membrane is the same, there is NO NET MOVEMENT of water across the membrane.

This last condition is known as EQUILIBRIUM. While individual molecules may migrate across the membrane at equilibrium, movements into the cell are balanced by movements out of the cell. Again, there is no NET movement of water at equilibrium.

Two major parameters influence water potential: solute concentration (Ψ_s solute potential) and hydrostatic pressure (Ψ_p pressure potential). They essentially have an additive effect:

$$\Psi = \Psi_s + \Psi_p$$

THE EFFECT OF SOLUTES

The presence of solutes dissolved in water lowers the water potential. Obviously then, water moves from an area where the solute concentration is lower to an area where the solute concentration is higher. Distilled water is the purest water (highest possible Ψ_s 0 MPa) and water with dissolved salts has lower water potential (Ψ_s < 0, that is negative).

The exact solute potential of a solution is determined by the van't Hoff equation: $\Psi_s = -CiRT$. Here C is the molar concentration of the solutes, i is the osmotic coefficient, R is the gas constant, and T is the absolute temperature. RT has the value of 2.27 liter MPa mol⁻¹ at 0 C and is 2.48 liter MPa mol⁻¹ at 25 C. The value of i is 1 for molecules that do not dissociate in solution and can be 2 or more for molecules that completely dissociate (such as salts). Recall that C is in units of moles liter⁻¹. The term Ci is the OSMOLAR CONCENTRATION or OSMOLARITY of the solution. The osmolarity of a complex solution is the sum of the osmolarity of each of the solute components. As an exercise for homework, calculate the osmolarity of the solutes in the complete nutrient solution in the Mineral Nutrition project.

THE EFFECT OF PRESSURE

Water tends to move away from pressure. That is, pressure INCREASES the water potential. At sea level, the pressure of the atmosphere is defined as 0 MPa. Negative pressures (Ψ_p < 0) indicate that a partial vacuum (tension) is present; this is seldom observed in living plant tissues or cells, but may be common in dead cells of the xylem. When a vacuum is present, water will rush into it from an area of normal (0 MPa) pressure. Since a living plant cell has a cell wall, the movement of water into an already-filled cell can produce positive pressure (Ψ_p > 0). This is frequently called TURGOR PRESSURE. Since cells are normally completely filled with cytoplasm (cell volume ≥ 1.0), most adjustments needed to come to equilibrium with an external water potential influence pressure potential prior to significant changes in cell solute concentration (see Höfler diagram at http://plantphys.info/Plant_Physiology/copyright/hofler.jpg or Fig 3.10 in the textbook).

In this exercise, you will observe osmotic events in potato slices by 1) determining how much cells swell or shrink when they come to equilibrium with different concentrations of an impermeant solute, sorbitol; 2) measuring the rate of water movement as cells approach equilibrium with different concentrations of sorbitol; and 3) determining solute concentration of potato sap.

I. EQUILIBRIUM OSMOTIC EFFECTS

Since a cell membrane is largely impermeable to sorbitol compared to its permeability to water, cells placed in HYPEROSMOTIC (solute concentration greater in the solution than inside the cell) sorbitol solutions will lose water to the solution. Cells placed in HYPOSMOTIC (solute concentration less in the solution than inside the cell) sorbitol solutions gain water from the solution. Cells placed in ISOSMOTIC (solute concentration of the solution equal to that inside the cell) sorbitol solutions will neither gain nor lose water. The gain or loss of water, measured here by weight, will be an indicator of net osmosis in potato slices.

1. Use the 1 M sorbitol stock solution and distilled water to make 50 ml each of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 M sorbitol solutions in seven separate cups. Label each cup. Keep the cups covered with plastic wrap.
2. The instructor will use a mandoline to cut uniform sticks of potato tuber tissue. You will be provided with 21 uniform sticks from this one potato. The remainder of the potato will be wrapped in plastic for further use (Part III).
3. Cut the sticks to a uniform length (approx. 3 cm), blot dry, and weigh each group of three sticks to 0.01 g. The group should be in the range of 3 grams. Record the weight in the laboratory record in a table according to their respective solutions. Be careful to keep track of which sticks are to go into each solution! Record the data with equal caution!
4. Place the sticks into their assigned solutions for 20 minutes. Occasionally and regularly swirl the covered cups during this incubation period.
5. At the end of the incubation period, remove each group of tissue sticks, blot them between clean, dry, paper towels, and weigh them to 0.01 g. Work quickly and carefully. Record the weight in the proper place on the table in the laboratory record. Immediately return the sticks to their respective solution.
6. Repeat steps 4 and 5 until there is no net change in ANY group of sticks.
7. At equilibrium, note which of the tissue sticks seem "crispy" and which seem "limp" and record your general observations in the laboratory record.
8. At home, make the calculations and plots described in the laboratory record. **Do not do this now!**
9. Keep the tissue sticks in their 0.0 M and 0.5 M sorbitol solutions. Discard the other sticks, their solutions, and the five empty cups. You may then proceed with Part II.

II. OSMOTIC KINETICS

The time course (kinetics) of water movement will now be followed more carefully. You will use the sticks and solutions kept from the previous section (above). These have reached equilibrium with their respective solutions. At the initial time, you will purposely move the sticks equilibrated in the 0.5 M solution to the 0.0 M solution and vice versa. You will then repeat the data collection at five-minute intervals.

1. Quickly but carefully blot and weigh the sticks from each equilibration solution. Record this initial weight in the laboratory record under the heading for the OTHER solution.
2. Put the sticks in their NEW solution (the one under which their initial weight is recorded). Incubate with swirling for five minutes.
3. Remove the sticks, blot, and reweigh. Return the sticks to the solution for another five minutes. Note: do not count the time when the sticks are out of their solutions as incubation time.
4. Repeat step 3 until there is no further weight change in either of the two groups of sticks.
5. AT HOME, make the calculations and plots requested in the laboratory record.

III. OSMOTIC POTENTIAL DETERMINATION

The Osmette™ osmometer measures the osmolarity of a solution by determining the freezing point depression of a sample. A small amount of liquid in an Eppendorff centrifuge tube is cooled rapidly in the instrument while its temperature is recorded. When the temperature drops well below freezing (supercooling) a small rod in the sample is vibrated to allow ice crystals to form. The temperature of the liquid stabilizes at the freezing point. The freezing point measured is electronically converted to osmolarity which appears as a series of digits on the instrument's readout. The conversion factor used by the instrument is $0.54 \text{ mol } ^\circ\text{C}^{-1}$. We could have determined this manually by similar manipulation in an ice bath and observation of an ASTM 52C BUTA BPR thermometer (range: -10 to +5 C)

The Wescor™ vapor pressure osmometer measures the osmolarity of a solution by determining the vapor pressure depression by thermocouple hygrometry. The sample chamber is cooled rapidly to a temperature below the dew point of the sample. As water condenses on the thermocouple, the temperature of the thermocouple stabilizes at the dew point. The depression of this dew point is used to determine the osmolality of the sample. A sample of 1 millimole kg^{-1} would have a depression of $0.00031 \text{ } ^\circ\text{C}$. The instrument must be very accurate in measuring temperature!

1. Chop an undamaged part of the remainder of the potato, grind it to a pulp in a mortar, and squeeze out a small volume of sap into an Eppendorff centrifuge tube. If necessary this can be capped and frozen for thawing and later use. **Do not add any water to this liquid at any time!**
2. The liquid should be centrifuged for 2 minutes in the microcentrifuge.
3. As directed by Dr. Koning, use the micropipette to transfer the required volume into the osmometer. Record the resulting osmolarity in the laboratory record.
4. Use this information to perform the calculations requested in the laboratory record.

Laboratory Record for Osmosis in Plant Cells

Name _____

Date _____

PART 1. EQUILIBRIUM OSMOTIC CONDITIONS

Record the initial weight for each stick set you observed in the fourth column of Table 1. Keeping track of each set of sticks in its respective solution, record subsequent weights across the chart.

Table 1. Effect of various sorbitol concentrations on water uptake of potato sticks.

Volume of 1 M Sorbitol (ml)	Volume of Water (ml)	Final Concentration (M)	Weight of Sticks at			
			0 minutes (g)	20 minutes (g)	40 minutes (g)	60 minutes (g)
30	20	0.6				
25	25	0.5				
20	30	0.4				
15	35	0.3				
10	40	0.2				
5	45	0.1				
0	50	0.0				

AT HOME, perform the necessary calculations to fill in Table 2.

- The sorbitol water potential is calculated from the van't Hoff equation: $\Pi_s = -CiRT$. Recall that $RT = 2.48$ liter MPa mol⁻¹ at 25 C. For sorbitol $i = 1$. The pressure potential of an open solution is 0 MPa by definition.
- The relative cell volume is calculated by dividing the equilibrium weight by the initial weight for each stick from the data in Table 1.
- Since sorbitol is impermeant, we correct the internal solute potential at equilibrium simply by dividing the initial solute potential by the relative cell volume. This accounts for the swelling or shrinking of the cells and its effects on osmolarity of the cytoplasm.

The initial solute potential is determined using the data taken from the osmometer. The osmolarity _____ is divided by 0.85 (=_____) to take into account the extracellular liquid which has negligible osmolarity. This liquid comprises about 15% of the total volume of potato sap. The corrected osmolarity is then multiplied by -2.48 (=_____ MPa) to determine the initial tissue solute potential. This number can now be divided by the relative cell volumes to convert them to equilibrium tissue solute potentials.

- d. The tissue pressure potential at equilibrium is now easily calculated by applying the water potential formula: $\psi = \psi_s + \psi_p$. Since you are calculating equilibrium conditions, you may assume that the water potential of the cells is identical to the water potential of the sorbitol bathing solution. You have just calculated in c above, the tissue solute potential at equilibrium, so now you simply solve for pressure potential.
- e. You should note that in cases where the initial tissue solute potential is greater than the sorbitol solute potential, the pressure potential should be 0 MPa. How well do your calculations meet this expectation? How did the relative "limpness" of the sticks compare with pressure potential?

Table 2. Calculations summary for the osmotic conditions at equilibrium.

Sorbitol Concentration (M)	Sorbitol Water Potential (MPa)	Equilibrium Relative Cell Volume	Equilibrium Tissue Solute Potential (MPa)	Equilibrium Tissue Pressure Potential (MPa)
0.6				
0.5				
0.4				
0.3				
0.2				
0.1				
0.0				

- f. Make the following plots from Table 2 above:
- (1). Equilibrium relative cell volume vs sorbitol concentration
 - (2). Equilibrium tissue (=sorbitol) water potential vs relative cell volume
 - (3). Equilibrium tissue solute potential vs relative cell volume
 - (4). Equilibrium tissue pressure potential vs relative cell volume

An overlay of plots 2, 3, and 4 is called a _____.

PART 2. RATE OF OSMOSIS TO EQUILIBRIUM

Record the new initial and subsequent stick set weights for the stick sets exchanged from their equilibrium solutions in Table 3 below.

Table 3. Data summary for weight changes after moving potato sticks from an equilibrium medium to a different medium.

Treatment	Weight of Sticks at						
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
0.0 to 0.5 M							
0.5 to 0.0 M							

AT HOME, perform the necessary calculations to fill in Tables 4 and 5.

- a. Fill in Table 4 by dividing each weight in Table 3 by the appropriate value for initial stick weight from Table 1. Divide numbers in the top row of Table 3 by the t=0 weight from row 7 of Table 1. Divide numbers in the bottom row of Table 3 by the t=0 weight from row 2 of Table 1.

Table 4. Relative cell volume after moving potato sticks from an equilibrium medium to a different medium.

Treatment	Relative Cell Volume at						
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
0.0 to 0.5 M							
0.5 to 0.0 M							

- b. Plot these relative cell volumes from Table 4 against time after changing from the equilibrium medium to the new medium. Draw the best smooth curve through the points for each exchange direction.
- c. Calculate the gross permeability of the sticks from the equation: $Q = k(\Delta\Psi)$. Q is the rate of water transport, k is the gross permeability, and $\Delta\Psi$ is the difference between the water potential of the tissue and the water potential of the sorbitol medium.

(1). Initial Q is calculated as the initial slope of the two curves you plotted in b above.

(2). The value for $\Delta\Psi$ for initial times is simply the difference in potential between the equilibrium solution and the new solution.

(3). Gross permeability is calculated by dividing Q by $\Delta\Psi$.

Table 5. Calculation summary for estimating gross permeability from the rate of osmosis.

	Q = Rate of Water Transport	Relative Cell Volume	Sorbitol Water Potential	Tissue Water Potential	Potential Difference = $\Delta\Psi$	Gross Permeability
0.0 to 0.5 M						
0.5 to 0.0 M						

- d. Geometric factors such as the shape of cells, dimensions of the tissue stick, proximity and connectedness of intercellular spaces, etc. and the frequency of agitation play a role in determining the rate of water transport and affect the calculation of gross permeability. We have ignored these factors for this simple experiment.

PART 3. OSMOTIC POTENTIAL DETERMINATION

Osmette/Wescor result: _____ M

Questions for thought:

1. To what degree can the results obtained in this exercise with tissue sticks be applied to intact plants?
2. Too much fertilizer applied to the soil will drastically reduce plant growth. Other than chemical toxicity, what could be a contributing factor to this reduction?
3. Most plants could not survive growing in a salt marsh. Certain species are able to cope with this quite well. How would you explain this?
4. Many seeds have water-impermeable seed coats. How do these aid in dormancy, and how might germination be possible?
5. If a leaf is wilted, what is the pressure potential of an average cell? What is the condition of the cytoplasm?
6. If you were to attempt to culture plant protoplasts (cells lacking their cell wall) in vitro, what molarity would your incubating medium have to be in order to avoid bursting the cells or shrinking them drastically?