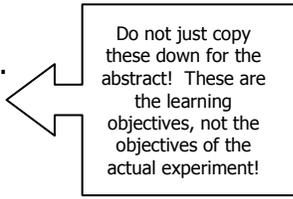


LAB 04 – Diffusion and Osmosis

Objectives:

- Describe the physical mechanisms of diffusion and osmosis.
- Understand the relationship between surface area and rate of diffusion.
- Describe how molar concentration affects the process of diffusion.
- Predict cell outcomes when changing the concentration of solute in a solution in which the cell is suspended.
- Determine the molar concentration of sucrose in a plant cell.



Do not just copy these down for the abstract! These are the learning objectives, not the objectives of the actual experiment!

Introduction:

Many aspects of the life of a cell depend on the fact that atoms and molecules are constantly in motion (the concept of kinetic energy). This kinetic energy results in molecules bumping into and rebounding off each other and moving in new directions. One result of this molecular motion is the process of diffusion.

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes, including the plasma and organelle membranes, are selectively permeable. Membranes are phospholipid bilayers containing embedded proteins. The phospholipid fatty acids limit the movement of water because of their hydrophobic characteristics.

The cellular environment is aqueous, meaning that the solvent is water, in which the solutes, such as salts and organic molecules, are dissolved. Water may pass freely through the membrane by osmosis or through specialized protein channels called aquaporins. Most ions move through protein channels, while larger molecules, such as carbohydrates, are carried by transport proteins.

The simplest form of movement is diffusion, in which solutes move from an area of high concentration to an area of low concentration; diffusion is directly related to molecular kinetic energy. Diffusion does not require energy input. The movement of a solute from an area of low concentration to an area of high concentration requires energy input in the form of ATP and protein carriers called pumps.

Water moves through membranes by diffusion; this process is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high water concentration) and low solute concentration to areas of low potential (low water concentration) and high solute concentration. In walled cells, osmosis is affected not only by the solute concentration but also by the resistance to water movement in the cell by the cell wall. This resistance is called turgor pressure (the physical pressure exerted on the cell).

The terms *hypertonic*, *hypotonic*, and *isotonic* are used to describe solutions separated by selectively permeable membranes.

- A hypertonic solution has a higher solute concentration and a lower water potential as compared to the other solution; therefore, water will move into the hypertonic solution through the membrane.
- A hypotonic solution has a lower solute concentration and a higher water potential than the solution on the other side of the membrane; water will move down its concentration gradient into the other solution.
- Isotonic solutions have equal water potential.

Understanding Water Potential (Ψ_w)

In non-walled cells, such as animal cells, the movement of water into and out of a cell is affected by the relative solute concentration on either side of the plasma membrane. As water moves out of the cell, the cell shrinks; if water moves into the cell, it swells and may eventually burst or lyse. In walled cells, including fungal and plant cells, the presence of a cell wall prevents the cells from bursting as water enters; however, pressure builds up inside the cell and affects the rate of osmosis.

Water potential predicts which way water diffuses through plant tissues and is abbreviated by the Greek letter psi (Ψ_w). Water potential is the free energy per mole of water and is calculated from two major components: (1) the solute potential (Ψ_s) – also called the osmotic potential (sometimes shown as Ψ_π) – is dependent on solute concentration, and (2) the pressure potential (Ψ_p), which results from the exertion of pressure – either positive or negative (tension) – on a solution.

Water Potential = Pressure Potential + Osmotic Potential

$$\Psi_w = \Psi_p + \Psi_\pi$$

Water moves from an area of higher water potential or higher free energy to an area of lower water potential or lower free energy. Water potential measures the tendency of water to diffuse from one compartment to another compartment.

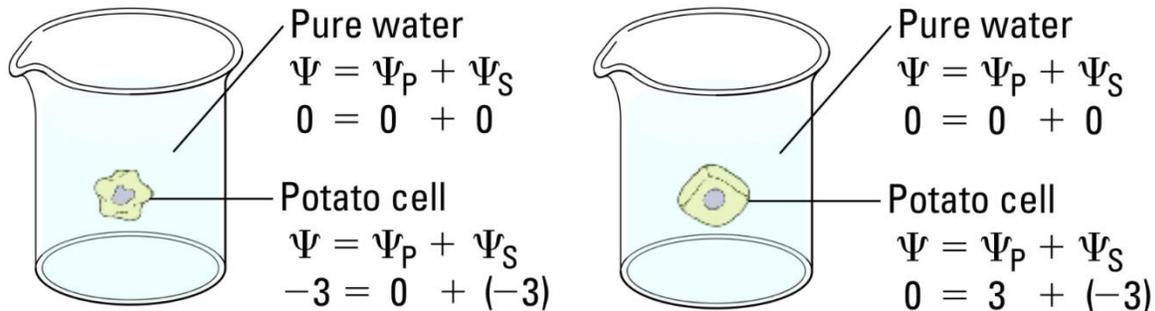
The water potential of pure water in an open beaker is zero ($\Psi_w = 0$) because both the solute and pressure potentials are zero ($\Psi_\pi = 0$; $\Psi_p = 0$). An increase in positive pressure raises the pressure potential and the water potential. The addition of solute to the water *lowers the solute potential* and therefore **decreases** the water potential. This means that a solution at atmospheric pressure has a negative water potential because of the solute.

The solute potential (Ψ_π) = $-iCRT$, where i = the ionization constant, C = the molar concentration (a.k.a. osmolarity), R = the pressure constant ($R = 0.0831 \text{ liter} \cdot \text{bars/mole} \cdot \text{K}$), and T = the temperature in K ($273 + ^\circ\text{C}$).

A 0.15 M solution of sucrose at atmospheric pressure ($\Psi_p = 0$) and 25°C has an osmotic potential of -3.7 bars and a water potential of -3.7 bars. A bar is a metric measure of pressure and is the same as 1 atmosphere at sea level. A 0.15 M NaCl solution contains 2 ions, Na^+ and Cl^- (where sucrose stays as one particle); therefore $i = 2$, and the water potential = -7.4 bars.

When a cell's cytoplasm is separated from pure water (e.g. distilled water) by a selectively permeable membrane, water moves from the surrounding area, where the water potential is higher ($\Psi_w = 0$), into the cell, where water potential is lower because of solutes in the cytoplasm (Ψ_w is negative). It is assumed that the solute is not diffusing (Figure 1a). The movement of water into the cell causes the cell to swell, and the cell membrane pushes against the cell wall to produce an increase in pressure. This pressure, which counteracts the diffusion of water into the cell, is called turgor pressure.

Over time, enough positive turgor pressure builds up to oppose the more negative solute potential of the cell. Eventually, the water potential of the cell (not just osmotic potential!) equals the water potential of the pure water outside the cell (Ψ_w of cell = Ψ_w of pure water = 0). At this point, a dynamic equilibrium is reached and net water movement ceases (Figure 1b)



Figures 1a-b: Plant cell in pure water. The water potential was calculated at the beginning of the experiment (a) and after water movement reached dynamic equilibrium and the net water movement was zero (b).

If solute is added to the water surrounding the plant cell, the water potential of the solution surrounding the cell decreases. If enough solute is added, the water potential outside the cell is then equal to the water potential inside the cell, and there will be no net movement of water. However, the solute concentrations inside and outside the cell are not equal because the water potential inside the cell results from the combination of both the turgor pressure (Ψ_P) and the solute pressure (Ψ_S), as shown in Figure 2.

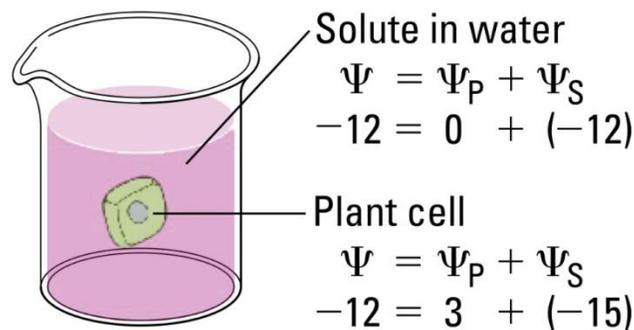


Figure 2: Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not equal.

If more solute is added to the water surrounding the cell, water will leave the cell, moving from an area of higher water potential to an area of lower water potential. The water loss causes the cell to lose turgor pressure. A continued loss of water will cause the cell membrane to shrink away from the cell wall, and the cell will plasmolyze.

Name: _____

Calculate the solute potential of a 0.1 M NaCl solution at 25°C.

If the concentration of NaCl inside the plant cell is 0.15 M, which way will the water diffuse if the cell is placed into the 0.1 M NaCl solution? HINT: Draw a diagram like **Figure 2**.

This concept can be reviewed on pages 765-767 in your textbook.

This investigation consists of three parts. In Procedure 1, students use models of cells to study the relationship of surface area and volume and their impact on the rate of diffusion. In Procedure 2, they create models of living cells out of selectively permeable dialysis tubing to explore the rate of osmosis under different sized osmotic gradients. Students finish by observing osmosis in Procedure 3 by using living cells to determine the osmolarity of different types of apples.

Procedure:**PART I – Surface Area vs. Rate of Diffusion**

1. Label one test tube 0.1 M HCl and another 0.1 M NaOH.
2. Using separate DPTPs (Disposable Plastic Transfer Pipettes), place 2 mL of phenolphthalein into each of two test tubes.
3. Answer questions #1 – 3 below.
4. Using a dull knife or thin strip of hard plastic, cut three cubes of 3% agar treated with phenolphthalein of the following sizes:
 - a. 1 cm³
 - b. 2 cm³
 - c. 3 cm³
5. Determine the surface area and volume for each of these 'cells'. Enter values in **Table 1** on page 6.
6. Answer question #4 on page 6.
7. Fill the bottom of a large petri dish with 0.1 M HCl. Carefully place each of the 3% agar cubes into the dish.
8. Record the time it takes for each cube to completely lose its color in **Table 1**.
9. Using a dull knife or thin strip of hard plastic, make two 12 cm³ rectangles of 3% agar treated with phenolphthalein of the following dimensions:
 - a. 3 cm X 4 cm X 1 cm
 - b. 3 cm X 2 cm X 2 cm
 - c. 6 cm X 2 cm X 1 cm
10. Determine the surface area and volume for each of these 'cells'. Enter values in **Table 2** on page 6.
11. Answer question #5 on page 6.
12. Use the same large petri dish filled with 0.1 M HCl. Carefully place each of the 3% agar rectangles into the dish.
13. Record the time it takes for each rectangle to completely lose its color in **Table 2**.

Questions:

- 1) Which solution is an acid? _____
- 2) Which solution is a base? _____
- 3) What color is the indicator phenolphthalein in each of the above?

- 4) If you put each of the cubes into a solution, which cube would that solution completely diffuse through the fastest? How do you explain the different rates of diffusion?

Results:**PART I****Table 1: The relationship between the surface-area-to-volume ratio and diffusion.**

| Length of Cube Side | Surface Area (cm ²) | Volume (cm ³) | Surface Area: Volume Ratio | Time Until Complete Diffusion |
|---------------------|---------------------------------|---------------------------|----------------------------|-------------------------------|
| 1 cm | | | | |
| 2 cm | | | | |
| 3 cm | | | | |

- 5) If you put each of the rectangles into a solution, which rectangle would that solution completely diffuse through the fastest? How do you explain the different rates of diffusion?

Table 2: The relationship between surface area and diffusion.

| Dimensions of 12 cm ³ Rectangle (cm) | Surface Area (cm ²) | Volume (cm ³) | Surface Area: Volume Ratio | Time Until Complete Diffusion |
|---|---------------------------------|---------------------------|----------------------------|-------------------------------|
| 3 X 4 X 1 | | 12 | | |
| 3 X 2 X 2 | | 12 | | |
| 6 X 2 X 1 | | 12 | | |

Procedure:**PART II – Effects of Osmotic Potential Differences Across a ‘Membrane’**

1. Obtain six, ~20 cm strips of pre-soaked dialysis tubing.
2. Tie off one end of each piece with ‘twisty ties’ to form 6 bags.
3. Pour 20 mL of each of the following sucrose solutions into separate bags:
 - a. 0.0 M sucrose – distilled water
 - b. 0.2 M sucrose
 - c. 0.4 M sucrose
 - d. 0.6 M sucrose
 - e. 0.8 M sucrose
 - f. 1.0 M sucrose
4. Remove some of the excess air from each bag and tie off with ‘twisty ties’.
5. Rinse each bag to remove sucrose from the string and outside surface.
6. Carefully blot the outside of each bag and record the initial mass of each bag in **Table 3**.
7. Place each bag in one of three 250 mL beakers (or plastic cup) and fill with 200 mL of distilled water. Label each beaker with your group number and molar concentration of bag.
8. Let stand for 20 minutes, then remove the bags and carefully blot each.
9. Determine the mass and record in **Table 3** for the solutions you were assigned.
10. Record data of percent change in mass in your corresponding group number in **Table 4** (on the class computer as well).
11. Using Excel, incorporate onto a graph the mean, standard deviation, and error bars of the class results.

(HINT: http://www.uvm.edu/~jleonard/AGRI85/spring2004/Standard_Error_Bars_in_Excel.html)

Results:**PART II****Table 3: Dialysis Bag – Individual Group Data**

| Contents of beaker | Initial Mass | Final Mass | Mass Difference | % Change in Mass* |
|--------------------|--------------|------------|-----------------|-------------------|
| 0.0M sucrose | | | | |
| 0.2M sucrose | | | | |
| 0.4M sucrose | | | | |
| 0.6M sucrose | | | | |
| 0.8M sucrose | | | | |
| 1.0M sucrose | | | | |

$$*\text{Percent Change in Mass} = \frac{(\text{Final Mass}) - (\text{Initial Mass})}{\text{Initial Mass}} \times 100$$

Table 4 – Dialysis Bag – Class Data

| | Percent Change in Mass of Dialysis Bags | | | | | | | | Mean | Standard Deviation | Standard Error |
|---------------------|---|---------|---------|---------|---------|---------|---------|---------|------|--------------------|----------------|
| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | | | |
| 0.0M sucrose | | | | | | | | | | | |
| 0.2M sucrose | | | | | | | | | | | |
| 0.4M sucrose | | | | | | | | | | | |
| 0.6M sucrose | | | | | | | | | | | |
| 0.8M sucrose | | | | | | | | | | | |
| 1.0M sucrose | | | | | | | | | | | |

Procedure:**PART III – Determining the Osmolarity of Different Apples**

1. Obtain 100 mL of each of the sucrose solutions and pour each solution into a separate, labeled 250 mL beaker (or plastic cup).
2. Use a cork borer (approximately 5 mm in inner diameter) to cut 24 apple cylinders. (Make sure that these are solid and have no cross-cuts. This will keep the surface area the same for all of the cylinders.) Cut each cylinder to segments 3 cm in length. Remove any skin found on the cylinders.
3. Determine the mass of 4 of the cylinders together, and record in **Table 5.***. Put these 4 cylinders into one of your sucrose solutions.
4. Do the same for 4 other cylinders and place in your second sucrose solution.
5. Do the same for the remaining cylinders (in groups of 4) and place in the remaining sucrose solutions.
6. Cover the beakers with plastic wrap.
7. Let stand overnight.
8. The next day, record the temperature of the sucrose solutions in **Table 5.***.
9. Remove the cores from one of the beakers, blot them gently – don't soak up water from "inside" the cylinders – on paper towel and determine their combined mass. Do the same for your two other beakers.
10. Record the final masses and calculate percent change in **Table 5.***.
11. Record all data of percent change in mass for each apple variety in **Table 6**.

* - you will have one of these tables for **EACH** type of apple you are testing

Results: (PART III)**Table 5.* – Apple Core Results – Variety:** _____

| Contents of beaker | Temperature | Initial Mass | Final Mass | Mass Difference | % Change in Mass¶ |
|--------------------|-------------|--------------|------------|-----------------|-------------------|
| 0.0M sucrose | | | | | |
| 0.2M sucrose | | | | | |
| 0.4M sucrose | | | | | |
| 0.6M sucrose | | | | | |
| 0.8M sucrose | | | | | |
| 1.0M sucrose | | | | | |

$$\text{¶Percent Change in Mass} = \frac{(\text{Final Mass}) - (\text{Initial Mass})}{\text{Initial Mass}} \times 100$$

Table 6 – Apple Core Results

| | Percent Change in Mass of Apple Cores | | | | |
|---------------------|--|--|--|--|--|
| | Apple Variety | | | | |
| | | | | | |
| 0.0M sucrose | | | | | |
| 0.2M sucrose | | | | | |
| 0.4M sucrose | | | | | |
| 0.6M sucrose | | | | | |
| 0.8M sucrose | | | | | |
| 1.0M sucrose | | | | | |

Hints For Your Lab Report:

The Introduction section of your lab should have your hypotheses for Part III of this investigation. You should do a little research on each apple variety that your group chose for this investigation. Cite accordingly. Do not hypothesize for Parts I and II as those sections are not part of this lab write-up. We will use the data from Part III to find the osmolarity of each type of apple cells.

The Results section of your lab report will include (but not be limited to!):

- All data tables your group utilized in Part III – properly formatted, using Excel.
- Graphs of each apple type properly labeled and given an appropriate title. Regression analysis (best fit line) should be used—along with R^2 value!
 - Remember, the **I**ndependent variable is what **I**, the **I**nvestigator is changing (x-axis). The dependent variable is the results—what you are measuring as a result of your change (y-axis).
- Your determination of the osmolarity of each apple type. This is where the mass of the potato cores would not change – use your regression analysis for this calculation. Use the formula for the straight line that best fits your data. The point at which this line crosses the X-axis represents the molar concentration of sucrose with a water potential equal to the potato tissue water potential—therefore, it will be where there is a 0% change. Be sure to include this very important calculation as well as stating your answer in the Results section!
- Using $\Psi_{\pi} = -iCRT$, find the osmotic potential of each type of apple. Set up calculation and solve using the correct units! Be sure to state this in the Results section!
 - $i = 1$ (ionization constant for sucrose)
 - C = osmolarity of apple variety
 - $R = 0.0831 \text{ L} \cdot \text{bars/mole} \cdot \text{degree K}$ (pressure constant)
 - T = temperature in Kelvin (Celsius + 273)