

LAB #4: Diffusion and Osmosis

Objectives:

- Describe the physical mechanisms of diffusion and osmosis.
- 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 actual experiment objectives!

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.

Diffusion is the random movement of molecules from an area of higher concentration to an area of lower concentration. **Osmosis** is a special case of diffusion. It is the diffusion of water through a selectively permeable membrane from a region of higher water potential to a region of lower water potential until an equilibrium is reached. Water potential is the measure of free energy of water in a solution.

In this lab, you will first work with a cell model using dialysis tubing to represent your cell. You will fill dialysis tubing with various sucrose concentrations and immerse them in distilled water. In part 2 of this lab, you will be working with a real cell system—potato cells. You will immerse cores of potato in different molar concentrations of sucrose to determine the water potential of potato cells.

Water potential is the free energy of water, water will always move from an area of higher water potential to an area of lower water potential (high free energy to low free energy).

Water potential has 2 components: **osmotic potential**—which is dependent on solute concentration and **pressure potential**—which results from the exertion of pressure either positive or negative on a solution. This can be reviewed on pages 765-767 in your textbook.

$$\text{Water Potential} = \text{Pressure Potential} + \text{Osmotic Potential}$$

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

The water potential of pure water in a beaker at STP is **0** because both osmotic and pressure potentials are 0. The addition of a solute to water lowers the osmotic potential (makes Ψ_w more negative and, therefore, lowers the water potential).

When dealing with plant cells like those found in a potato, osmotic potential of a cell is lowered as a result of more solute dissolved in its cytoplasm. When placed in a pure water situation, the cells are now hypertonic to the environment. The water potential in the beaker is higher than in the cell. Water will diffuse into the cell until the pressure potential equalizes. As a result, the cell will swell.

Conversely, if solute is added to the water of the beaker so that the water potential is higher in the cell than the beaker, the cell is said to be hypotonic to its surroundings and water will diffuse out of the cell until the pressures are equal. Water basically move from a higher water potential to a lower one.

Procedure: (PART I—work in groups of 4)

PRE-LAB: Students will make the various sucrose solutions beforehand.

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 25 mL of each of the following sucrose solutions into separate bags:
 - a. 0.0M sucrose—distilled water
 - b. 0.2M sucrose
 - c. 0.4M sucrose
 - d. 0.6M sucrose
 - e. 0.8M sucrose
 - f. 1.0M sucrose
4. Remove excess air from each bag and tie off with 'twisty ties'.
5. Rinse each bag under tap water 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 1.1**.
7. Place each bag in one of three 250 mL beakers (or cup if that is the only option) and fill with distilled (or tap if that is the only option) water to the 200 mL mark. Label beaker with appropriate information.
8. Let stand for 20 minutes.
9. At the end of 20 minutes, remove the bags and carefully blot each.
10. Determine the mass and record in **Table 1.1** for the solutions you were assigned.
11. Record data of percent change in mass in **Table 1.2** (both in your tables and also on the class computer).

Results: (PART I)**Table 1.1—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				

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

Procedure: (PART II—work in groups of 4)

PRE-LAB: Students will make the various sucrose solutions and potato cores beforehand.

1. Obtain 100 mL of each of the sucrose solutions and pour each solution into a separate, labeled 250 mL beaker (or cup if that is the option).
2. Use a cork borer (approximately 5 mm in inner diameter) to cut 24 potato 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 2.1**. 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 2.1**.
9. Remove the cores from one of the beakers, blot them gently 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 2.1**.
11. Record data of percent change in mass in **Table 2.2** (both in your tables and also on the class computer).

Results: (PART II)**Table 2.1—Potato Core Results—Individual Group Data**

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					

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

Hints For Your Lab Report:

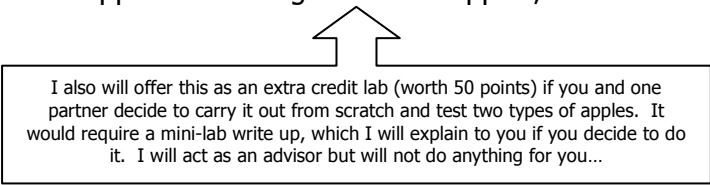
The Introduction section of your lab should have (but not be limited to—refer to your lab manual for all necessary components) your hypotheses for Part I of this investigation. Do not hypothesize for Part II. We will use the data from Part II to find the osmolarity of the potato core cells to be used in our calculations of the Results section.

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

- All data tables (both individual and class—total of 4), properly formatted, using Excel.
- Graphs of each table (total of 4) 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 the sucrose solution in which the mass of the potato cores would not change—use your individual group data for this calculation. To find this, use the regression analysis to draw 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 in the Results section!
- Using $\Psi_{\pi} = -iCRT$, find the osmotic potential of the potato cells. Set up calculation and solve using the correct units! Be sure to include this in the Results section!
 - $i = 1$ (ionization constant for sucrose)
 - $C =$ osmolarity (from your graph of individual group data)
 - $R = 0.0831$ L bars/mole degree K (pressure constant)
 - $T =$ temperature in Kelvin (Celsius + 273)

The Discussion section of your lab report will include your interpretation of the results for both parts (make sure to discuss the significance of the R^2 value, the answers to the following questions, and any other information as referenced in your Lab Guide):

1. Describe the relationship between the increase of mass and the molarity of sucrose within the dialysis bags.
2. Predict what would happen in an experiment if all the dialysis bags were placed in a 0.6M sucrose solution instead of distilled water. Draw your “predicted” line on your graph for Table 1.2 and label it “**0.6M prediction**”.
3. If a potato is allowed to dehydrate by sitting in the open air, would the water potential of the potato cells increase or decrease? Explain using the water potential formula from the introduction.
4. If a plant cell has a lower water potential than its surrounding environment and if pressure equals 0, is the cell hypertonic or hypotonic to its environment? What would have to happen for the contents of the cell to become isotonic to the environment?
5. How could this lab technique be applied in finding out which apples, Macintosh or Delicious are sweeter?



I also will offer this as an extra credit lab (worth 50 points) if you and one partner decide to carry it out from scratch and test two types of apples. It would require a mini-lab write up, which I will explain to you if you decide to do it. I will act as an advisor but will not do anything for you...

Table 2.2—Potato Core Results—Class Group Data

	Percent Change in Mass of Potato Cores								Class Average
	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									