Overview
In this lab you will:
1) Investigate the processes of diffusion and osmosis in a model membrane system, and
2) Investigate the effect of solute concentration on water potential as it relates to living plant tissues.

Objectives
Before doing this lab you should understand:
A) The mechanisms of diffusion and osmosis and their importance to cells
B) The effects of solute size and concentration gradients on diffusion across selectively permeable membranes
C) The effects of a selectively permeable membrane on diffusion and osmosis between two solutions separated by the membrane
D) The concept of water potential
E) The relationship between solute concentration and pressure potential and the water potential of a solution
F) The concept of molarity and its relationship to osmotic concentration

After doing this lab you should be able to do:
A) Measure the water potential of a solution in a controlled experiment
B) Determine the osmotic concentration of living tissue or an unknown solution from experimental data
C) Describe the effects of water gain or loss in animal and plant cells
D) Relate osmotic potential to solute concentration and water potential

Introduction
Many aspects of a cell's life depend on the fact that atoms and molecules are constantly in motion (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 in which the random movement of molecules results in the net movement of molecules from an area of greater concentration to an area of lesser concentration. Barring outside forces, eventually a dynamic equilibrium will be reached in which the concentration of molecules is approximately equal and no net movement of molecules will occur from one area to another.

Osmosis is a special case of diffusion. Osmosis is the diffusion of water through a selectively permeable membrane (one that allows for diffusion of select solutes and water) from a region of greater water potential to a region of lesser water potential until an equilibrium is reached. Water potential is a measure of the free energy of water in a solution.

Diffusion and osmosis do not suffice to explain all the movement of molecules or ions into and out of cells. In these cases the movement is usually caused by active transport, a process in which cells use carrier proteins and energy to move substances through the cell membrane, often against a concentration gradient (i.e., from a region of lower concentration to a region of higher concentration).

In this laboratory activity you will investigate the processes of diffusion and osmosis in a model membrane system and the effect of solute concentration on water potential in living plant tissues. You will try to determine whether different chemical substances pass through a cell membrane with equal ease, if the rate of water diffusion is related to concentration differences and how water potential is measured. The model cell will be made from dialysis tubing, a cellophane material that is selectively permeable. Although it is nonliving, dialysis membrane has several properties of living cell membranes. Potatoes will be used to represent living plant tissues.

Exercise 1A: Diffusion
In this experiment you will measure diffusion of small molecules through dialysis tubing, an example of a selectively permeable membrane. Small solute molecules and water molecules can move freely through a selectively permeable membrane, but larger molecules will pass through more slowly, or perhaps not at all. The movement of a solute through a selectively permeable membrane is called dialysis. The size of the minute pores in the dialysis tubing determines which substances can pass through the membrane.

A solution of glucose and starch will be placed inside a bag of dialysis tubing. Distilled water will be placed in a beaker, outside the dialysis bag. After 30 minutes have passed, the solution inside the dialysis tubing and the solution in the beaker will be tested for glucose and starch. The presence of glucose will be tested with Benedict’s solution. The presence of starch will be tested with Lugol’s solution.

Part 1A. Diffusion
1. In this part of the lab investigation you will answer the question, how does particle size affect diffusion through a selectively permeable membrane? Propose a hypothesis and rationale for this question and make an if/then prediction statement for this experiment.

2. Take a soaked piece of dialysis tubing from the beaker at your table and rub the tubing between your thumb and forefinger to open it. Make a dialysis tubing bag by tying one end into a tight knot.

3. Place approximately 15 ml of the glucose and starch solution in the bag. Tie the open end of the bag leaving sufficient space for expansion of the bag’s contents. Check the bag for leaks and rinse it thoroughly with tap water.

4. Fill a 250 mL beaker two-thirds full with distilled water. Add approximately 4 mL of Lugol’s solution to the distilled water.

4. Record the color of the solution in a data table similar to Table 1 below.

8. After 30 minutes, test the liquid in the beaker and the cell’s contents for starch and for glucose. Compare your observations with other teams. Record your results.

Table 1.

<table>
<thead>
<tr>
<th>Initial Contents</th>
<th>Presence of Starch</th>
<th>Presence of Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Bag (15%glucose &amp; 1% Starch)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beaker (H₂O &amp; IKI)</td>
<td></td>
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</tbody>
</table>

Exercise 1B: Osmosis

In this experiment you will use dialysis tubing to investigate the relationship between solute concentration and the movement of water through a selectively permeable membrane by the process of osmosis.

When two solutions have the same concentration of solutes, they are said to be isotonic to each other. If the two solutions are separated by a selectively permeable membrane, water will move between the two solutions, but there will be no net change in the amount of water in either solution.

If two solutions differ in the concentration of solutes that each has, the one with more solute is hypertonic to the one with less solute. The solution that has less solute is hypotonic to the one with more solute. These words can only be used to compare solutions.

Now consider two solutions separated by a selectively permeable membrane. He solution that is hypertonic to the other must have more solute and therefore less water. At standard temperature an pressure, the water potential of the hypertonic solution is less than the water potential of the hypotonic solution, so the net movement of water will be from the hypotonic solution into the hypertonic solution.

Part 1B. Osmosis

1. In this part of the lab investigation you will answer the question, how do different concentration gradients affect the rate of osmosis? Propose a hypothesis and rationale for this question and make an if/then prediction statement for this experiment.

2. Take a soaked piece of dialysis tubing from the beaker at your table and rub the tubing between your thumb and forefinger to open it. Make a dialysis tubing bag by tying one end of the tube into a knot.

3. Pour 15-25 ml of one of the following solutions (assigned by your teacher) into the bag.

   a) 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 most of the air from the bag by drawing it between two fingers. Tie the open end of the bag leaving sufficient space for expansion of the bag’s contents. *(The solution should fill only about one-third to one-half of the bag.)*

5. Check the bag for leaks, rinse it thoroughly with tap water, and carefully dry it with a paper towel.

6. After carefully zeroing a balance, weigh and record the initial mass of your bag.

7. Place the dialysis tubing bag into a labeled, 250 ml beaker and add distilled water until it is nearly full. Let the beaker stand for 30 minutes.

8. At the end of 30 minutes remove the bag from the beaker, carefully blot it dry and determine its mass. Record your measurement.

9. Collect class data for all the bags and record.

<table>
<thead>
<tr>
<th>Table 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bag Contents</td>
</tr>
<tr>
<td>Distilled Water</td>
</tr>
<tr>
<td>0.2 M Sucrose</td>
</tr>
<tr>
<td>0.4 M Sucrose</td>
</tr>
<tr>
<td>0.6 M Sucrose</td>
</tr>
<tr>
<td>0.8 M Sucrose</td>
</tr>
<tr>
<td>1.0 M Sucrose</td>
</tr>
</tbody>
</table>

10. Calculate percent change in mass for each bag using the following formula:

\[
\text{Percent Change in Mass} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100
\]

11. Make a best-fit line graph of the class data. (Remember to put the independent variable on the x-axis and the dependent variable on the y-axis.)

**Exercise 1C: Water Potential**

In this part of the exercise you will use potato cores placed in different molar concentrations of sucrose in order to determine the water potential of potato cells. Botanists use the term water potential when predicting the movement of water into or out of plant cells. Water potential is abbreviated by the Greek letter psi (Ψ) and it has two components: a physical pressure component (pressure potential Ψ_p) and the effects of solutes (solute potential Ψ_s).

**Part C. Determining Water Potential in Real Cells**

1. In this part of the lab investigation you will answer the question, *what is the water potential of fresh potato cells?* In order to fully understand this procedure, please read the Appendix entitled, *Water Potential*, found at the end of the lab instructions.

2. You will be assigned the same solution of sucrose (or distilled water) that you used in Part B. Pour 40 ml of the assigned liquid into a labeled 50 ml beaker.

3. Use a cork borer to cut three potato cylinders. Cut each cylinder to approximately 4 cm in length. *Do not include any skin on the cylinders.*

4. Determine the mass of the three cylinders together and record the mass. Put the three cylinders into the beaker, being certain that the cores are completely covered with liquid. Cover the beaker with plastic wrap to
prevent evaporation and let stand over night. (Be sure to label the beaker.)

5. On the next day, (at lunch or after school) measure and record the temperature of the liquid in your beaker. (It will be used later in calculations of osmotic potential.)

6. Remove the potato cores from your beaker, blot them gently on a paper towel, and determine their mass.

7. Record the final mass and calculate percent change as in Part B. Add your data to the class data table.

8. Collect class data for all the solutions, calculate averages for each molarity, and record the data. Make a best-fit line graph of the class data.

9. To determine the osmolarity of the sucrose solution in which the mass of the potato cores does not change, draw the straight line that best fits your class data. The point at which this line crosses the x-axis represents the molar concentration of sucrose with a water potential that is equal to the potato cells water potential. At this concentration there is no net gain or loss of water.

10. The osmotic potential of this sucrose solution can be calculated using the following formula:

\[ \Psi = - ICRt \]

Where \( I \) = the ionization constant (for sucrose this is 1 because sucrose does not ionize in water); \( C \) = osmotic molar concentration (determined above); \( R \) = pressure constant (handbook value \( R = 0.0831 \) liter bars/mole K); and \( T \) = temperature K (273 + °C of solution). Calculate and record.

11. Knowing the osmotic potential of the solution and the pressure potential of the solution is zero, calculate and record the water potential of the potato cells.

<table>
<thead>
<tr>
<th>Bag Contents</th>
<th>Temperature</th>
<th>Initial Mass</th>
<th>Final Mass</th>
<th>Percent Change in Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>0.2 M Sucrose</td>
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<td>0.8 M Sucrose</td>
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<td></td>
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<tr>
<td>1.0 M Sucrose</td>
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Discussion

1. In Part A, why were you directed to rinse your cell before placing it in the beaker of water?

2. In Part A, did starch diffuse through the cell membrane? What is your experimental evidence?

3. In Part A, did glucose diffuse through the cell membrane? What is your experimental evidence?

4. Restate your hypothesis for Part A and explain why your experimental evidence does or does not support it.
5. In Part B, what is the relationship between the change in mass and the molarity of sucrose in the dialysis bags?

6. In Part B, what was the function of the distilled water bag?

7. Restate your hypothesis for Part B and explain why your experimental evidence does or does not support it.

8. Explain your results from Part B using the concept of free energy.

9. In the graph of Part C, the point at which the line crossed the x-axis represents the molar concentration of sucrose with a water potential that is equal to the potato cells water potential. What adjective is used to describe this solution?

10. If your potato cores were allowed to dehydrate in the open air before testing, would the water potential of the cells change? Explain.

11. If a plant cell has a lower water potential than its surrounding environment and if pressure is equal to zero, is the cell hyperosmotic (in terms of solute concentration) or hypoosmotic to its environment? Explain.