Diffusion and Osmosis Lab
Activity A: Diffusion

Objectives

- Use dialysis tubing to model diffusion across the cell membrane.
- Investigate the influence of solute concentration on osmosis

Background to Activity A

In the last unit, you learned about the molecules that are necessary to sustain life. In Unit 2 you will see how cells use these molecules to carry out homeostasis, metabolism and protein production. In order for cells to use macromolecules, they must have a way to get the molecules into the cell. All molecules enter the cell through the cell membrane.

The cell membrane is a cell’s interface with its surroundings. In one sense, this membrane must function as a barrier: it must keep together in one bundle the enzymes, DNA, and metabolic pathways that make life possible. The cell membrane must also function as a gateway: waste products must be discharged through it and essential materials (oxygen, water, carbohydrates, proteins, etc.) must enter through it. A membrane that allows some molecules to pass through while blocking the passage of others is said to be semipermeable. Molecules pass through the cell-membrane either through processes that require the cell to expend energy (active-transport), or through processes driven by the kinetic (thermal) energy of molecules (passive transport).

In these lab activities, you will investigate the passage of materials through a semipermeable membrane by passive transport. The membrane you will use, dialysis tubing, is semipermeable because it has submicroscopic holes through it. Molecules are in constant random motion. By chance, a molecule’s motion may move it toward the membrane (figure 1). If it collides with the membrane wall, it rebounds. If its motion takes it toward a pore, it may pass through the pore. If the molecules pass through the membrane, the concentration (or amount) of molecules will increase. As the concentration of solute molecules increases, they displace some of the water molecules, forcing them to pass through the membrane to the other side. Thus, there will be a higher concentration of water molecules on the opposite side of the membrane. More water molecules are available to collide with the membrane on the side having the higher concentration of water. Thus, although water molecules will move in both directions across the membrane, more will move from the side having the higher concentration to the side having lower concentration. The movement of molecules from areas of higher concentration to areas of lower concentration is called diffusion.

Materials

Dialysis tubing, plastic cup, glucose/starch solution, distilled water, iodine-potassium iodine (IKI) solution, dropping pipet, glucose test strips, funnel. Caution: IKI solution can irritate the skin, mouth, and eyes, and can stain skin or clothing

Introduction
In Activity A, you will explore the diffusion of different molecules through dialysis tubing, a semipermeable membrane. When iodine-potassium iodide (IKI) reacts with starch, it becomes part of the starch molecule and is removed from solution. We can easily observe this chemical reaction because the starch solution changes from a milky white color to blue-purple when it reacts with the IKI.

Pre-Lab Questions
1. What would a color change indicate when you combine the starch and iodine in step 3?

2. Make a prediction: After 30 minutes has passed, do you expect to observe a color change in the cup or inside of the dialysis tubing? If so, predict what color the solutions will be.

   Inside of the cup: ____________________________________________________________

   Inside of the dialysis bag: ______________________________________________________

Procedure
Setting up a Control Test
1. Pour 5 mL of distilled water into the test tube labeled IKI. Add approximately 5 mL of IKI solution to the water and mix well. Record the solution color in Table 1.
2. Pour 5 mL of distilled water into the test tube labeled Starch. Add approximately 5 mL of Starch solution to the water and mix well. Record the solution color in Table 1.
3. Pour 5 mL of distilled water into the test tube labeled Starch + IKI. Add approximately 5 mL of Starch solution to the water and mix well. Add approximately 5 mL of IKI solution to the water and mix well. Record the solution color in Table 1.

Diffusion Test
4. Obtain a piece of dialysis tubing that has been soaked in water. The tubing should be soft and pliable. Roll the tubing between your thumb and index finger to open it. Close one end of the tube by knotting it or tying it off with string. This will form a bag.
5. Using a small funnel, pour 15 mL of glucose/starch solution in the dialysis bag. Smooth out the top of the bag, running it between your thumb and index finger to expel the air. Tie off the open end of the bag. Leave enough room in the bag to allow for expansion. Record the initial color of the solution in the bag in table 2.
6. Pour 160-170 mL of distilled water into a plastic cup. Add approximately 4 mL of IKI solution to the water and mix well. Record the initial color of the solution in the cup in table 2.
7. Immerse the dialysis bag in the solution in the cup. Make sure that the portion of the bag that contains the glucose/starch solution is completely covered by the solution in the cup at all times.
8. Record the color of the solution in the cup in Table 2.
9. Wait 30 minutes. While waiting, read through and discuss the analysis questions with your partner.

Data & Observations, Activity A

Table 1: Control Test

<table>
<thead>
<tr>
<th></th>
<th>IKI</th>
<th>Starch</th>
<th>Starch + IKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color of Solution</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Diffusion Test

<table>
<thead>
<tr>
<th></th>
<th>Inside the Dialysis Tubing</th>
<th>Inside the Cup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Color of Solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final Color of Solution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Results, Activity A
1. Indicate on Figure 2 the initial locations (inside or outside of the bag) of all the kinds of molecules that are available for diffusion through the dialysis membrane.

![Initial Location of molecules]
2. What were the final colors of the solutions in the dialysis tubing and in the cup? Label the final locations of each molecule on Figure 2.

3. Based on your observations of IKI and starch solutions, what conclusions can you draw about the solution in the dialysis tubing and in the cup?

4. Compare your results with your predictions. Do you find any conflicts that would cause you to revise your predictions? If so, explain.

5. What molecules were present during this activity? Does this activity account for all of the molecules that you listed? If not, what data could have been collected to show the net direction of diffusion of this unaccounted for molecule or molecules?

6. What does your data tell you about the sizes of the molecules relative to the pore size of the dialysis tubing?

**Diffusion and Osmosis Lab**

**Activity B: Osmosis**

**Objectives**
- Investigate the concept of water potential in relation to water movement into or out of plant cells

**Background to Activity B**
The diffusion of water molecules across a semipermeable membrane is termed **osmosis**. A process that depends upon random motion might seem inefficient, but so many water molecules are involved and they move so fast. It is estimated that a red blood cell floating in blood plasma gains an amount of water equal to 125 times its own volume every second. It also loses the same amount of water each second, all by osmosis. This occurs because the concentration of solutes in the blood plasma is the same as the concentration of solutes in red blood cells. Solutions that have the same solute concentration are **isotonic**. If we took a sample of whole blood and added salt to the plasma, increasing its solute concentration, the plasma becomes **hypertonic** to the solution in the red blood cells, and the cells lose water and shrink. If we add water to the blood plasma, decreasing its solute concentration, the plasma becomes **hypotonic** to the solution in the red blood cells. The cells gain water, swell, and may even burst.

**Materials**
Dialysis tubing, plastic cups, distilled water, funnel, sucrose solutions, balance, calculator (optional).

**Introduction**
In Activity B, you will investigate the influence (if any) of solute concentration on the net movement of water molecules through a semipermeable membrane. The solute you will use is sucrose (cane or table sugar) in the following molar concentrations:

- 0.0 M (distilled water), 0.2 M, 0.4 M, 0.6 M, 0.8 M, & 1.0 M
**Pre-Lab Questions**

1. Write a hypothesis that this experiment is designed to test.

2. What are you measuring in this experiment?

3. List at least three variables or factors (other than the one listed in your answer to #2) that could influence the outcome of this experiment. Briefly describe the method of control used for each of these variables.

<table>
<thead>
<tr>
<th>Variables Influencing Results</th>
<th>Method of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Ex: variations in tube size and mass</em></td>
<td>Calculating the change in mass excludes the mass of the tubing from the results.</td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure**

Complete the following steps for each sucrose solution that you are assigned to test.

1. Pour ~150 mL of distilled water into a plastic cup. Label the cup with the concentration of sucrose that you will test.

2. Obtain a piece of dialysis tubing that has been soaked in water. The tubing should be soft and pliable. Roll the tubing between your thumb and index finger to open it. Close one end of the tube by knotting it or tying it off with a piece of string. This will form a bag.

3. Using a small funnel, pour ~15 mL of sucrose solution into the dialysis bag. Smooth out the top of the bag, running it between your thumb and index finger to expel the air. Tie off the open end of the bag. Leave enough room in the bag to allow for expansion.

4. Dry the bag gently with a paper towel and then determine its mass. Record this as the initial mass in Table 2.

5. Immerse the dialysis bag in the water in the cup. Make sure that the portion of the bag that contains the sucrose solution is completely covered by the water in the cup at all times. Wait 30 minutes before continuing to the next step.

6. After 30 minutes, remove the bag from the cup and dry it gently with a paper towel. (It should be as dry as it was before you measured the initial mass.) Mass the bag and record the final mass in Table 2. Finally, determine the change in mass of the bag and record this data in Table 3.

**Data**

**Table 3: Osmosis Activity**

<table>
<thead>
<tr>
<th>Contents in Dialysis Bag</th>
<th>Initial Mass (g)</th>
<th>Final Mass (g)</th>
<th>Change in Mass (g)</th>
<th>Your % ΔMass</th>
<th>Class Average of % ΔMass</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 M sucrose (distilled water)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.2 M sucrose</td>
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<tr>
<td>0.4 M sucrose</td>
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<tr>
<td>0.6 M sucrose</td>
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<tr>
<td>0.8 M sucrose</td>
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<tr>
<td>1.0 M sucrose</td>
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</table>

**Analysis of Results, Activity B**

Answer the following questions on a separate piece of paper.

1. Calculate the percent change in mass (% ΔMass) using the following formula (show your work!).

\[
\% \Delta \text{Mass} = \frac{\Delta \text{Mass}}{\text{Mass}_{\text{initial}}} \times 100
\]

Record your results in Table 3.
2. Obtain and record the class averages for change in mass and percent change in mass in Table 3.

3. What does the change in mass indicate?

4. Graph the percent change in mass for your group data and the class averages. Title the graph and supply the following information:
   a. The independent variable is _________________________________.
   b. The dependent variable is _________________________________.
   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

8. Explain any differences between the graph for your group and the graph of the class average.

9. On the basis of your data and graph, has this experiment adequately tested the variable you listed under #5?

10. Do your results support your hypothesis, refute it, or require that you modify it? Explain.

11. On the basis of your results, write a statement that expresses the relationship of solute concentration and direction of net movement of water molecules in osmosis.

12. In which, if any, of the experimental setups were the solutions in the bag and outside of the bag isotonic to each other?

13. If the experimental setup specified that only distilled water be used to fill the dialysis bag and that the sucrose solutions be used to fill the cup, how would that change your results?

14. When you drink a glass of water, most of it is absorbed by osmosis through cells lining your small intestine. Drinking seawater can actually dehydrate the body. How?