Lab 3. Diffusion and Osmosis in Selectively Permeable Membranes

Prelab Assignment

Before coming to lab, read carefully the introduction and the procedures for each part of the experiment, and then answer the prelab questions on the first two pages of the report pages. Be prepared to hand in the prelab questions just before the start of your scheduled lab period.

Goals of this Lab

After completing this lab exercise you should be able to.....

• Distinguish between and describe diffusion and osmosis.
• Explain why diffusion and osmosis are important to cells.
• Describe what is meant by selectively permeable and explain the role of a selectively permeable membrane in osmosis.
• Determine the effects of concentration on diffusion and osmosis.
• Describe the effects of hypertonic, isotonic, and hypotonic solutions on animal and plant cells.
• Explain how the presence of a cell wall affects osmotic behavior of plant cells.

Introduction

All living things must obtain certain materials from their environment. For example, most animal cells obtain nutrients, oxygen, and water from their surroundings. In addition, they must get rid of waste materials. This exchange of substances between the cell and its environment is critical to survival and is dependent upon these materials being dissolved in water. Water is a great environment for life. Without it, life as we know it is impossible. If, as suspected, life in our solar system exists only on earth, it is probably because ours is the only planet known to possess liquid water on its surface.

The cytoplasm and extracellular environment of living cells are aqueous. The cytoplasm of living cells is composed of water (~70% to 80% water), which is the solvent or dissolving agent, and numerous dissolved solutes (e.g. salts, sugars, amino acids, vitamins, etc.). Virtually all substances entering and leaving cells are dissolved in water. The combination of a solvent and dissolved solute(s) is a solution.

To perform their functions, cells must maintain a steady state in the midst of an ever-changing environment. This constancy is maintained by the regulation of the movement of solutes into and out of the cell. To achieve this control, a delicate membrane composed of a phospholipid bilayer that contains embedded and surface proteins surround cells. Cell membranes (also called plasma membranes) can distinguish different substances, slowing or inhibiting the movement of some while allowing others to pass through. Because not all substances penetrate the membrane equally well, cell membranes are said to be selectively permeable (or differentially permeable), allowing some substances to pass through easily, while completely or partially excluding others.

Although there are several methods by which solutes may enter or leave cells, the most common is diffusion. Diffusion is the movement of a substance (e.g. molecules and ions) from a region of higher concentration to one of lower concentration. Diffusion is a passive process, meaning that it occurs without the expenditure of energy. The energy driving diffusion comes only from the intrinsic kinetic energy found in all atoms, ions, and molecules. If nothing obstructs the movement, a solute will diffuse until it spreads itself out uniformly and reaches a dynamic equilibrium.

So far, we’ve only described the movement of solutes across membranes. However, water (the solvent) also moves across cell membranes. The movement of water across a selectively permeable membrane from a region where water is highly concentrated to a region where its concentration is lower is known as osmosis.

Osmosis is a special kind of diffusion, the diffusion of water. In osmosis water always moves by diffusion across a selectively permeable membrane from a hypotonic solution (low solute concentration, high water conc.) to a hypertonic solution (high solute concentration, low water conc.). A hypertonic solution has a high solute concentration (and therefore a low concentration of water) relative to another solution. This is because when a solute is dissolved in water, its molecules (or
ions) fill the space previously occupied by water molecules, thus reducing the concentration of water. On the other hand, a hypotonic solution has a low solute concentration (and thus a high concentration of water) relative to another solution because in the absence of large amounts of solute there is more space available for water molecules. Hence, osmosis can also be defined as the diffusion of water molecules across a selectively permeable membrane from a dilute solution (low solute conc. = hypotonic) to one that is concentrated (high solute conc. = hypertonic).

A cell placed into a hypertonic solution will shrink due to the loss of water. If too much water is lost, cell death may result. On the other hand, if a cell is placed in a hypotonic solution, water will enter the cell, causing it to swell and possibly burst. A cell placed in an isotonic solution, one that has the same solute concentration as another solution, the cytoplasm in this case, will not gain or lose water because water molecules will enter and leave the cell at equal rates.

A. More water enters the cell than leaves the cell: There is a net movement of water from the hypotonic environment (lower solute conc., higher water conc.) to the hypertonic cytoplasm (high solute conc., low water conc.).

B. Crenation: More water leaves the cell than enters the cell: There is a net movement of water from the hypotonic cytoplasm to the hypertonic extracellular environment.

Figure 1. The two diagrams illustrate the process of osmosis, the diffusion of water across a selectively permeable membrane from a hypotonic to a hypertonic solution. The membrane is impermeable to the solute in these examples.

The difference in concentration of like molecules in two regions (e.g. on each side of a cell membrane) is called a concentration gradient. Diffusion and osmosis take place down concentration gradients, that is, from an area of high concentration to an area of low concentration. Over time, the concentration of the solvent and solute particles becomes equally distributed, and the gradient ceases to exist. At this point the system is said to be at equilibrium. At equilibrium solute and solvent continue to move randomly in all directions, but with no net change in their concentration.

This lab investigation will introduce you to the principles of diffusion and osmosis and study their underlying causes and some of the factors regulating these processes. Through a series of demonstrations and exercises, the principles of moving substances across membranes will be studied. Each exercise will reveal a different yet related aspect of transportation into and out of cells. Bear in mind that these principles are put into practice continually by cells trying to function and maintain homeostasis in an ever changing environment. Whether the organism is a single-celled protist, like a euglena, or a multi-celled animal, like a Homo sapiens, each cell needs to move solute molecules and water into or out of its cells or run the risk of cellular death or at the minimum, disruption of cellular function.

See pages 6 and 7 for figures that illustrate the concepts of diffusion and osmosis!
Overview of the procedure
This lab activity consists of three activities.

- **Activity 1** involves three pre-set up demonstrations that allow you to investigate molecular movement (diffusion and/or osmosis) through a semi-permeable membrane, a gel and living potato cells.
- **Activity 2** involves your team setting up four experimental setups to examine diffusion and/or osmosis through a non-living semi-permeable membrane (dialysis tubing).
- **Activity 3** examines osmosis in animal (red blood cells) and plant cells (Spirogyra) as you place the cells in hypertonic, isotonic and hypotonic solutions and observe them with a microscope.

### Procedures

**Activity 1. Demonstrations of Molecular Movements through a semi-permeable membrane, a gel and a living membrane**

The following three demonstrations are used to show the process of molecular movement due to Brownian motion (the random movement of molecules). The first demo involves the movement of molecules through a semi-permeable membrane, the second is through a semi-solid agarose gel medium, and finally through a living membrane. Prior to attending lab write hypotheses on for each demonstration on page 2 of the prelab questions. During the lab period record on the Report Sheet your measurements and observations of the molecular movements and then answer the questions in the spaces provided.

**Demonstrations**

1. Examine each of the three demonstrations. Record your observations for each demonstration and answer the questions on the Report Sheet.

   - **Demonstration 1: Thistle-tube demonstration.** The glass thistle tube contains a concentrated sugar solution and a blue dye. Attached to the bottom of the glass thistle tube is a semi-permeable membrane (dialysis tubing). The thistle tube is then lowered into a beaker filled with water only. What happened to the level of the blue solution in the thistle tube? What principle does this demonstration illustrate? Explain fully on the report sheet.

   - **Demonstration 2: Movement in a Gel.** The Petri dish was filled with agarose gel onto which crystals of two different dyes were placed: *potassium dichromate* (orange dye; molecular mass = 194 amu) and *aniline blue* (blue dye; molecular mass = 738 amu). This demonstration involves molecular movement through a semi-solid medium. Measure the diameter in millimeters (mm) of the "halo" created as each dye moved through the gel. Are there differences in the rate and total movement of each dye? Why? What principle does this demonstration illustrate? Explain fully on the report sheet.

   - **Demonstration 3: Movement through living membranes of potato cells.** This demonstration shows the results after potato slices were left in different salt solutions for about one day. Feel the slices in each dish and explain what could have happened to the potato slices to make them feel the way they do. What principle does this demonstration illustrate? Explain fully on the report sheet.
Activity 2. Diffusion through a non-living membrane

The purpose of this part is to demonstrate the action of a differentially permeable membrane. Such action can then be applied to the study of transport mechanisms in living cells.

Materials

- 4 - 250 ml. Beakers
- 1 small funnel
- four moistened dialysis sacs
- 1 - 10ml graduated cylinder
- 8 pieces of string ~25 cm long
- scissors
- wax pencil
- laboratory balance

Procedure

1. Formulate a hypothesis about the movement of the various solutes and water across the dialysis sac membrane for the four dialysis sacs in figure 2 by completing table 2 on page 4 of the report sheet.

2. Obtain four 250ml glass beakers and number them 1 to 4 with a wax marker.
   a. Fill beakers 1 and 3 about three-fourths full with DI water.
   b. Fill beaker 2 three-fourths full with a 40% glucose solution.
   c. Fill beaker 4 three-fourths full with Lugols’ iodine solution (I₂KI).

3. Prepare the dialysis sacs one at a time. To open the cellulose tubing, first moisten it with tap water. Then rub one end between your thumb and forefinger and place a funnel into the open end. Use string to tie a tight leak proof double knot about 1 cm. from the closed end of the tubing.

4. Using a graduated cylinder and a funnel, fill Sac 1 with 10 ml. of 40% glucose solution. Press out the air, fold over the open end of the sac, and tie it securely with piece of string to form a leak proof sac. Cut off the excess twine and sac and discard it in a wastebasket. Rinse the tube under running tap water to remove any solution from the outside. Carefully blot the sac dry by rolling it on a paper towel. After blotting the sac dry, weigh it with a laboratory balance (on the side benches). Record the mass in grams in Table 3 of the Report Sheet and place it next to its corresponding beaker.

5. Using a clean (or rinsed thoroughly with DI water) graduated cylinder and funnel, fill Sac 2 with 10 ml. of 40% glucose solution. Repeat the same procedures as given in step 4 above.

6. Using a clean (or rinsed thoroughly with DI water) cylinder and funnel, fill Sac 3 with 10 ml. of 10% NaCl solution. Repeat the same procedures as given in step 4 above.

7. Using a clean (or rinsed thoroughly with DI water) cylinder and funnel, fill Sac 4 with 10 ml. of Starch solution. Repeat the procedures as given in step 4 above.

8. Place the sacs at the same time into their corresponding beakers ensuring that each sac is submerged in the solutions.

9. Allow the sacs to remain undisturbed in the beakers for 1 hour. After one hour you will remove each of the sacs, gently blot them dry, weigh them, and record the data in Table 3 of the Report Sheet.

Note! Do not squeeze the sacs during the blotting process.
Activity 2 (cont.)

The Chemical Analysis of the beaker and sac contents

Materials

<table>
<thead>
<tr>
<th>Test tube rack with 3 test tubes</th>
<th>Metal test tube holders</th>
<th>hot plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 ml beaker with boiling chips</td>
<td>Benedict’s solution</td>
<td>silver nitrate (AgNO₃)</td>
</tr>
<tr>
<td>Small plastic beaker</td>
<td>scissors</td>
<td></td>
</tr>
</tbody>
</table>

Procedure:

10. Test for Glucose in Sac 1 and Beaker 1: Fill a 600 ml beaker 2/3rds full of tap water. Add a few boiling chips to the beaker. Place the beaker on the hot plate, start the water boiling before the end of the hour of soaking (about ten minutes), add water as necessary, and bring the water to a rolling boil. **While your water is heating, obtain two test tubes and label them 1 and 2.**

   a. Place 10 drops of Benedict’s solution in each test tube.

   b. Open sac 1 with scissors and put the fluid into a small plastic beaker. **Place 10 drops of the solution from sac 1 into tube #1.**

   c. Place 10 drops of the solution from beaker 1 into tube #2.

   d. Use the metal test tube holders to place tubes 1 and 2 into the beaker of boiling water. Boil for up to 2 minutes and cool slowly. If a green, yellow, or rusty-red precipitate forms, the test is positive for glucose (Green: small amount; Yellow: medium amount; Rusty-red: large amount). If the solution remains the original blue color, the test is negative, which means that no glucose is present. Use your results to fill in the appropriate spaces in Table 4 on the Report Sheet.

11. Test for Salt in Sac 3 and Beaker 3: Take a 10 drop sample of beaker 3 solution and put it in a clean test tube. Add a drop or two of silver nitrate (AgNO₃) to the test tube. The appearance of a white precipitate or cloudiness indicates the presence of AgCl, which is formed by the reaction of AgNO₃ with NaCl. Use your results to fill in the appropriate spaces in Table 4.

12. Test for Starch and Iodine in Sac 4 and Beaker 4: Iodine is used to indicate the presence of starch. When iodine, which is a yellow color, comes in contact with starch it turns a dark blue or black. **After one hour** of soaking examine Sac 4 and beaker 4 and note where the iodine came in contact with the starch. Use this observation to fill-in the appropriate spaces in Table 4.

13. The Clean up

   a. Solutions from the beakers, test tubes, and sacs may be poured down the sink.

   b. Place used dialysis tubing in the wastebasket.

   c. When finished with the hot plate turn it off and unplug it. Leave the water and boiling chips in the 600 ml beaker on the hot plate to cool.

   d. Clean the test tubes using detergent and a test tube brush. Place the cleaned tubes upside down in a test tube rack by the back sink.

   e. Rinse and clean using soap and water all other glassware used, place them in the drying by the back sink.

   f. Return all other materials (i.e. wax pencils, scissors, etc.) to their proper locations on the supply table.

   g. Wipe down the surface of your lab counter with detergent to clean up any solutions that might have leaked or spilled.

14. Answer the analysis questions 6 through 11 on the report sheet only after having completing all parts of activity 3 and cleaning up!
Activity 3. Osmosis in Living Animal and Plant Cells

The purpose of this part is to demonstrate the action of a "living" semi-permeable membrane. How do things pass through a cells membrane? What precautions must a cell take to ensure homeostasis and maintain functions, while still being an active vibrant cell? Think of how the principles demonstrated here can be applied to the normal and abnormal environments cells can find themselves involved with and yet still can maintain their lives.

![Diagram of red blood cells and plant cells in isotonic, hypotonic, and hypertonic solutions](image)

**Figure 3.** Plant and animal cells (red blood cells) in isotonic, hypotonic and hypertonic solutions

**Materials**

<table>
<thead>
<tr>
<th>Compound Light Microscope</th>
<th>Microscope slides</th>
<th>Cover slips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples of Red Blood Cells</td>
<td>Samples of <em>Spirogyra</em></td>
<td>Solutions A, B, and C</td>
</tr>
</tbody>
</table>

**Procedure: Red Blood Cells and Osmosis**

1. Label four microscope slides A, B, C, and D.
2. Place 3 drops of blood on slide D with a cover slip and observe the shape of the red blood cells. Record your observations on the Report Sheet in **Table 6**.
3. Place 3 drops of solution A on slide A, add a cover slip. Place the slide on the stage and add a drop of blood to the edge of the cover slip. Capillary action should move the blood under the cover slip. Watch blood cells at the edge of the advancing blood as they meet solution A. Observe the shape of the red blood cells under low, medium and high magnifications. Record your observations in **Table 6**.
4. Repeat step 3 for solutions B and C.
5. **Clean up!** Wash the slides in soap and water, dry and place them on the bench coat next to the sink.
6. Answer questions 12 through 15 on the report sheet only after having completing all parts of activity 3 and cleaning up!
Activity 3 (cont.)

Procedure: Plant cells and Osmosis

1. Label four clean and dry microscope slides A, B, C, and D.
2. Place on slide D a few strands of Spirogyra with 3 drops of water from the jar from which the Spirogyra came and then carefully add a cover slip. Place the slide on a microscope stage and observe the Spirogyra cells at low and medium magnifications and record your observations in Table 7 of the Report Sheet.
3. Place 3 drops of solution A on slide A. Place a few strands of Spirogyra into solution A on the slide and then add a cover slip. Place the slide on a microscope stage and observe the Spirogyra cells at low and medium magnifications and record your observations in Table 7 of the Report Sheet.
4. Repeat step 3 for solutions B and C.
5. Clean up! Wash the slides in soap and water, dry and place them on the bench coat next to the sink.

Figure 4. Demonstration of simple diffusion of red dye molecules

- Net movement of dye is to the left side of membrane
- At equilibrium, dye equal on both sides of membrane and net movement stops
- Solution – contains solute (dye or other substance) and solvent (water)

Figure 5. Demonstration of osmosis