Diffusion Through Membranes

Diffusion is a process that allows ions or molecules to move from where they are more concentrated to where they are less concentrated. This process accounts for the movement of many small molecules across a cell membrane. Diffusion allows cells to acquire food and exchange waste products. Oxygen, for instance, might diffuse in pond water for use by fish and other aquatic animals. When animals use oxygen, more oxygen will diffuse to replace it from the neighboring environment. Waste products released by aquatic animals are diluted by diffusion and dispersed throughout the pond.

It is important to consider how the rate of diffusion of particles may be affected or altered.

- Diffusion may be affected by how steep the concentration gradient is. The direction that a diffusing molecule or ion might travel is random. While the particles are diffusing, is there a net movement from where they are concentrated to where they are less concentrated?
- Diffusion may be affected by other different, neighboring particles. For instance, if oxygen diffuses towards a single-celled pond organism at a certain rate, will that rate be altered by the presence of another type of molecule? Would the presence of other molecules block or enhance the diffusion of a molecule? Would the molecule’s rate be independent of particles that do not alter the concentration gradient?

One way to measure the rate of diffusion of ions is to monitor their concentration in solution over a period of time. Since ions are electrically charged, water solutions containing ions will conduct electricity. A Conductivity Probe measures the concentration of ions in a solution, but not the concentration of electrically neutral molecules. Salts, such as sodium chloride, produce ions when they dissolve in water. If you place a salt solution inside a selectively permeable membrane such as dialysis tubing, the salt ions can diffuse out of the tubing and into the surrounding water.

OBJECTIVES
In this experiment, you will

- Use a Conductivity Probe to measure the conductivity of various solutions.
- Study the effect of temperature on diffusion.
- Study the effect of concentration gradients on the rate of diffusion.
- Determine if the diffusion rate for a molecule is affected by the presence of a second molecule.

MATERIALS

| TI-83 Plus or TI-84 Plus graphing calculator | dialysis tubing, 2.5 cm × 12 cm |
| EasyData application | dropper pipet or Beral pipet |
| data-collection interface | scissors |
| Vernier Conductivity Probe | stirring rod |
| three 18 × 150 mm test tubes with rack | 5% sucrose (table sugar) solution |
| 1%, 5%, and 10% salt water | dental floss or clamp |
| 400 mL beaker | ring stand and utility clamp |
PROCEDURE

1. Set up the utility clamp, and ring stand as shown in Figure 1.

2. Turn on the calculator. Set the Conductivity Probe on the 0–2000 µS/cm position. Connect the Conductivity Probe, data-collection interface, and calculator.

3. Set up the EasyData for data collection.
   a. Start the EasyData application, if it is not already running.
   b. Select [File] from the Main screen, and then select New to reset the application.
   c. Select [Setup] from the Main screen, then select Time Graph...
   d. Select [Edit] on the Time Graph Settings screen.
   e. Enter 5 as the time between samples in seconds.
   f. Select [Next].
   g. Enter 12 as the number of samples and select [Next](data will be collected for 60 seconds).
   h. Select [OK] to return to the Main screen.

Part I Concentration gradients

4. Test whether different concentration gradients affect the rate of diffusion. To do this, three solutions of differing salt concentrations (1%, 5%, and 10%) will be placed in distilled water. Each salt solution will be placed in a dialysis tube and allowed to diffuse into the surrounding water. When salt diffuses, the conductivity of the water in the beaker will increase.

5. In Table 1, predict what you believe will happen in this set of experiments. How will the rate of diffusion change when a 10% salt solution is placed in contact with pure water compared to when a 1% salt solution is placed in contact with pure water?
6. Prepare the dialysis tubing. Obtain a wet dialysis tube and a dialysis tube clamp or a short length of dental floss. Using the clamp or floss, tie one end of the tube closed about 1 cm from the end, as in Figure 2.

7. Place a 1% salt solution into a section of dialysis tubing. To do this,
   a. Obtain about 15 mL of a 1% salt water solution in a test tube.
   b. Using a funnel or Beral pipet, transfer about 10 mL of the 1% salt water into the dialysis tube, as in Figure 2. **Note:** To open the tube, you may need to rub the tubing between your fingers.
   c. Tie off the top of the dialysis tube with a clamp or a new length of dental floss. Try not to allow any air into the dialysis tube. The tube should be very firm after it is tied or clamped. Trim off any excess dental floss extending more than 1 cm from either knot.
   d. Wash the outside of the tubing with tap water thoroughly, so that there is no salt water adhering to the tubing.

8. Place 300 mL of distilled or deionized water into a 400 mL beaker. Secure the Conductivity Probe with the utility clamp in the water filled beaker as shown in Figure 1.

9. Place the dialysis tube into the water. Be sure the tubing is submerged completely under the water. **Important:** Position the Conductivity Probe and dialysis tubing the same distance apart in each trial.

10. After stirring the solution for 15 seconds, select to begin data collection. Stir the solution slowly and continuously throughout the one-minute data collection period.

11. Data collection will stop after 60 seconds. Analyze the graph to determine the rate of diffusion for the curve of conductivity vs. time:
   a. Examine the graph and determine the most linear region.
   b. Use to move the flashing cursor to the first point of the region. Enter the initial conductivity, $C_i$, and initial time, $t_i$, in Table 2.
   c. Move the flashing cursor to the last point of the region. Enter the final conductivity, $C_f$, and final time, $t_f$, in Table 2.

12. Remove one of the clamps. If the dialysis tubing is tied off with floss, use a pair of scissors and carefully cut one of the dental floss knots and discard the floss. If you accidentally make a cut in the tubing, replace it.

13. Empty all of the liquid out of the dialysis tube. Squeeze the excess liquid out with your fingers. Rinse the Conductivity Probe with distilled water.

14. Select to return to the Main screen.

15. Store the data from the first run so that it can be used later. To do this:
   a. Select and then select Store Run.
   b. Select to store your latest data and overwrite the data in Lists 3 and 4 (L3 and L4).
16. Obtain 15 mL of a 5% salt solution in a test tube. Repeat Steps 7–15, substituting this 5% salt solution for the 1% solution. **Note:** After selecting [Start] to begin data collection, select [OK] to start collecting data. Your stored data will not be overwritten.

17. Obtain 15 mL of a 10% salt solution in a test tube. Repeat Steps 7–14, substituting this 10% salt solution for the 1% solution.

18. Graph all three runs of conductivity data on a single graph. To do this:
   a. Select [Adv] and then select L2, L3 and L4 vs L1.
   b. All three runs should now be displayed on the same graph. Each point of the 1% salt is plotted with a plus sign, each point of the 5% salt is plotted with a square, and each point of the 10% salt is plotted without a marker. Examine the graph closely and make a conclusion. Record your conclusion in Table 1.
   c. When finished with the graph, select [Main] to return to the Main screen.

19. (Optional) Transfer your data to a computer for later printing per your teacher’s instructions.

**Part II Effect of other molecules**

In this set of experiments you will measure the rate of diffusion of salt while it is in the presence of a non-conducting molecule. Since sugar does not form ions in solution, it should not conduct electricity. Therefore, sugar will be added to the water to determine whether it interferes with the diffusion of salt.

20. In Table 1, predict what you believe will happen in this set of experiments. Will the non-conducting sugar in the water block or reduce the rate of diffusion of salt? Explain your prediction.

**Test to determine if water or a sugar solution conducts electricity.**

21. Place about 100 mL of distilled or deionized water in a clean 400 mL beaker.

22. Test the conductivity of the water by placing a clean Conductivity Probe into it.

23. Record the conductivity value displayed on the calculator screen in Table 4.

24. Obtain 300 mL of a 5% sugar solution in a clean 400 mL beaker.

25. Test the conductivity of the 5% sugar solution by placing a clean Conductivity Probe into it. Record the conductivity value in Table 4.

**Test if 5% sugar interferes with the diffusion of a 5% salt solution.**

26. Repeat Steps 6–14, with the following changes:
   a. Substitute a 5% salt solution for the 1% solution in Step 7.
   b. Use 300 mL of 5% sugar water in place of the water in Step 8.
## DATA

### Table 1

<table>
<thead>
<tr>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I</td>
</tr>
<tr>
<td>Part II</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Point 1</th>
<th>Point 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label</td>
<td>$C_i$ ($\mu S$)</td>
</tr>
<tr>
<td>1% salt</td>
<td></td>
</tr>
<tr>
<td>5% salt</td>
<td></td>
</tr>
<tr>
<td>10% salt</td>
<td></td>
</tr>
<tr>
<td>5% sugar</td>
<td></td>
</tr>
</tbody>
</table>

Slope = $\frac{C_f - C_i}{t_f - t_i}$

### Part I

### Table 3: Summary of Data

<table>
<thead>
<tr>
<th>Salt concentration (%)</th>
<th>Rate of diffusion ($\mu S$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

### Part II

#### Table 4

<table>
<thead>
<tr>
<th>Solution</th>
<th>Conductivity ($\mu S$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>Sugar water</td>
<td></td>
</tr>
</tbody>
</table>

#### Table 5: Summary of Data

<table>
<thead>
<tr>
<th>Solution</th>
<th>Rate of diffusion ($\mu S$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% salt</td>
<td></td>
</tr>
<tr>
<td>5% salt / 5% sugar</td>
<td></td>
</tr>
</tbody>
</table>
PROCESSING THE DATA

1. Using Table 2, calculate the rate of diffusion for the three salt solutions tested. Record each calculated rate in Table 3.

2. Using Table 2, calculate the rate of diffusion for the 5% salt solution in the 5% sugar water. Record the rate of diffusion in Table 5. Record the rate of 5% salt solution in distilled water from Table 3 in Table 5.

QUESTIONS

1. What conclusion can you draw from the data in Table 3?

2. How did your conclusion compare to your prediction for Part I? Can you account for any differences?

3. If the rates in any of the three experiments varied in Part I, calculate how much faster each rate was compared to that for the 1% salt solution. For instance, if the rate of the 1% solution was 1 µS/s and the rate of the 10% solution was 5 µS/s, then the rate of diffusion for the 10% solution would be (5/1) five times the rate of the 1% salt solution.

4. Compare the conductivity of pure water with a sugar solution. How do you account for this?

5. What conclusion can you draw from the data in Tables 4 and 5?

EXTENSIONS

1. Make a plot of the rate of diffusion vs. the salt concentration. Using your plot, estimate the rate of diffusion of a 3% salt solution.

2. If the results of the experiments in Part I can be extrapolated to diffusion in living systems, how would a single-celled organism respond in an oxygen rich pond compared to an oxygen-poor pond? Explain.

3. Design an experiment to determine the effect of temperature on the diffusion of salt. Perform the experiment you designed.

4. Ectotherms are organisms whose body temperature vary with the surrounding environment. On the basis of your data from Extension Question 3, how do you expect the oxygen consumption of ectotherms to vary as the temperature varies? Explain.

5. If waste products of an aquatic single-celled organism were released into a pond, how would that affect the organism’s ability to obtain oxygen from the pond water? Explain how your data from Part II supports your answer.
Diffusion through Membranes

1. The student pages with complete instructions for data-collection using LabQuest App, Logger Pro (computers), EasyData or DataMate (calculators), and DataPro (Palm handhelds) can be found on the CD that accompanies this book. See Appendix A for more information.

2. If the water in your area is very soft, you may want to use tap water instead of distilled water. Test to see if the conductivity of the tap water is less than about 50 mg/L salt.

3. Provide each group with pre-cut, hydrated dialysis tubing. The tubing must be soaked in water for at least ten minutes prior to use. The tubing should be soft and flexible.

4. Use dialysis tubing clamps if at all possible, as this will speed things up greatly. If desired, use dental floss or string to tie off the dialysis tubing. The floss works exceptionally well. You may want to show students how to tie off the dialysis tubes.

5. Have students check their dialysis tubes for leakage. This should be done before each experiment. Leaky tubes should be replaced.

6. Any sugar may be used in Part II. Table sugar is inexpensive and readily available.

7. To prepare 5% sugar solution, add 50 grams of sugar to make one liter of solution (300 mL per group is needed).

8. To prepare 1% salt solution, add 10 grams of NaCl to make one liter of solution (15 mL per group is needed).

9. To prepare 5% salt solution, add 50 grams of NaCl to make one liter of solution (30 mL per group is needed).

10. To prepare 10% salt solution, add 100 grams of NaCl to make one liter of solution (15 mL per group is needed).

11. The stored conductivity calibration (for 0–2000μS/cm) works well for this experiment. The ionic concentration is approximately proportional to the conductivity of the solution.
SAMPLE RESULTS
The following data may be different from students’ results.

Part I

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt concentration (%)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

Part II

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Table 4: Summary of Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>Conductivity (mg/L)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>x</td>
</tr>
<tr>
<td>Sugar water</td>
<td>x</td>
</tr>
</tbody>
</table>

Diffusion through dialysis tubing of differing salt concentrations

ANSWERS TO QUESTIONS
Answers have been removed from the online versions of Vernier curriculum material in order to prevent inappropriate student use. Graphs and data tables have also been obscured. Full answers and sample data are available in the print versions of these labs.