DIFFUSION THROUGH MEMBRANES

STANDARDS
- 3.2.12.B.1
- 3.2.12.C.4

INTRODUCTION

Many aspects of the life of a cell depend on the fact that atoms and molecules have kinetic energy and are constantly in motion. This kinetic energy causes molecules to bump into each other and move 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 of those molecules to an area of lower concentration. For example, if one were to open a bottle of hydrogen sulfide (H₂S has the odor of rotten eggs) in one corner of a room, it would not be long before someone in the opposite corner would perceive the smell of rotten eggs. The bottle contains a higher concentration of H₂S molecules than the room does and therefore the H₂S gas diffuses from the area of higher concentration to the area of lower concentration. As long as there is a concentration gradient, where there is an area of higher concentration of H₂S molecules in the room, there will be net movement of the molecules. Eventually, dynamic equilibrium will reached; the concentration of H₂S will be approximately equal throughout the room and no net movement of H₂S will occur from one area to the other.

It is important to consider how the rate of diffusion of particles might be affected or altered. Diffusion may be affected by the steepness of the concentration gradient (the difference between the number of ions or molecules in one region of a substance and that in an adjoining region). The direction that a diffusing molecule or ion might travel in any particular direction is random. While the particles are diffusing, is there a net movement from where they are concentrated to where they are less concentrated? Diffusion might 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 if some other molecule suddenly surrounded the organism? Would the presence of other molecules block or enhance the diffusion of a molecule, or would the molecule’s rate be independent of particles that do not alter the concentration gradient?

In this lab, you will directly measure the movement of salt ions through a dialysis membrane into distilled water. The use of three concentrations of salt will allow for examination of how different concentration gradients affect the rate of diffusion. You will also determine if the presence of a second molecule affects the rate of salt diffusion through the dialysis membrane.
GUIDING QUESTIONS

- Are there ways to directly detect diffusion through a membrane?
- What is the effect of different concentration gradients on the rate of diffusion?
- Is the diffusion rate for a molecule affected by the presence of a second molecule?

MATERIALS

- LabQuest
- LabQuest App
- Vernier Conductivity Probe
- three 18 × 150 mm test tubes with rack
- 1%, 5%, and 10% salt water
- 400 mL beaker
- ring stand and utility clamp
- dialysis tubing, 2.5 cm × 12 cm
- dropper pipet or Beral pipet
- plastic wrap
- stirring rod
- 5% sucrose (table sugar) solution
- dental floss or clamp
- Tape & permanent marker

PROCEDURE

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

2. Set the selector switch on the side of the Conductivity Probe to the 0–2000 µS/cm range. Connect the Conductivity Probe to LabQuest and choose New from the File menu. If you have an older sensor that does not auto-ID, manually set up the sensor.
   a. To manually identify the sensor, stay in the Meter mode and select Sensors → Sensor Setup…..
   b. A sensor set-up screen will appear showing all the available probe ports. Select the channel that the sensor is plugged into (ex. CH1 for channel 1), and tap the arrow to the side of the channel box.
   c. A list of compatible probes will appear in alphabetical order. Scroll down the list and select “Conductivity” and “Conductivity 2000MICS”. Select OK to return to the Meter mode screen.
   d. A red box will now be in this window displaying a real time conductivity (µS/cm) reading in microsemens per centimeter (Fig. 2).

3. On the Meter screen, tap Rate. Change the data-collection rate to 0.2 samples/second and the data-collection length to 120 seconds.

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 (Fig. 3). To do this,
   a. Obtain about 15 mL of a 1% salt water solution in a test tube.
   b. Use a funnel or Beral pipet to 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.

8. Wash the outside of the tubing with tap water thoroughly, so that there is no salt water adhering to the tubing. Rest the filled tubing on a piece of plastic wrap until you are ready to start the experiment. On the bench below the tubing, use tape and a marker to indicate that this tube contains the 1% salt solution.

9. Repeat Step 7-8 for the 5% and 10% salt solutions. You will need to prepare 2 pieces of dialysis tubing with 5% salt solution, one for Part I and one for Part II of the lab. Do not forget to label which piece of tubing contains which percent salt solution.

10. Place 300 mL of distilled or deionized water into a 400 mL beaker. Perform the following steps in the order described.
    a. Secure the Conductivity Probe with the utility clamp in the water filled beaker as shown in Figure 1.
    b. Start the data collection by tapping the Start button.
    c. Place the 1% salt 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.
d. Begin stirring the distilled water in the beaker. Stir the solution slowly and continuously throughout the two-minute data collection period. Data collection will stop automatically after 120 seconds.

11. Perform a linear regression to calculate the rate of reaction. If the graph is not completely linear, tap and drag the stylus across a linear portion of the data. Gray shading on the graph will indicate the section of data that will be analyzed using the following steps.

   a. While still in the Graph mode, choose Curve Fit from the Analyze menu. 
   b. Select Linear for the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form 
      \[ y = mx + b \]
   c. Enter the absolute value of the slope, \( m \), as the diffusion rate for 1% salt solution in Table 2.
   d. Select OK.

12. Store the data from the first run by tapping the File Cabinet icon to the right of the screen. Remove the first dialysis tube from the beaker and dispose of it. Empty the water from the beaker.

13. Repeat Steps 10-12 for one of the 5% salt solution tubes and for the 10% salt solution tube. Do not forget to store each run by tapping the File Cabinet icon to the right of the screen. Record the rate of diffusion for each run in Table 2.

14. To view all three runs on the same graph, tap the gray Run 4 box at the right of the screen, and select All Runs from the drop down menu.

**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.

15. 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.

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

16. Go to the Meter mode by tapping the icon at the top left of the screen.
17. Place about 100 mL of water in a clean 400 mL beaker. Test the conductivity of the water by placing a clean Conductivity Probe. Let the reading in µS/cm settle.

18. Record the conductivity value of the water in Table 3. Empty the water from the beaker.

19. Obtain 300 mL of a 5% sugar solution and place it in the clean 400 mL beaker.

20. While still in the Meter mode, test the conductivity of the 5% sugar solution by placing a clean Conductivity Probe into it. Record the conductivity value in µS/cm in Table 3. Keep the 5% sugar solution in the beaker for the next part of the lab.

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

21. Return to the Graph mode by tapping the icon at the top of the screen. Repeat the procedure used in Part I (Steps 10-12), with the following changes:
   a. Use 300 mL of 5% sugar water in the beaker instead of distilled water.
   b. Use the second dialysis tube of 5% salt solution.
   c. Record the slope, \(m\), as the rate of diffusion in (µS/cm/s) in Table 4.

22. To view all four runs on the same graph, tap the gray Run 5 box at the right of the screen, and select All Runs from the drop down menu.

REFERENCES


Kelly Redding and David Masterman. Advanced Biology with Vernier. (2008) Diffusion through Membranes. Vernier Software & Technology; 13979 S.W. Millikan Way, Beaverton, OR pp. 1A-1to 1A-6(O_2), 1A-1T to 1A-3T.

CREDITS

Special thanks John Thompson and the AP Biology class (2009-10) of Neshannock High School, Neshannock Township, PA for testing, editing and reviewing this protocol. The lab was revised and adapted from the above references by Dr. Stephanie Corrette-Bennett.
DATA SHEET

Name: _______________________
Group: _______________________
Date: _______________________

DATA ANALYSIS

Table 1: Predictions

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

Part I:

Table 2: Rate of Salt Diffusion

<table>
<thead>
<tr>
<th>Salt Concentration (%)</th>
<th>Rate of Diffusion (µS/cm/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 3: Conductivity Readings

<table>
<thead>
<tr>
<th>Solution</th>
<th>Conductivity (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>5% sugar</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Comparison of Diffusion

<table>
<thead>
<tr>
<th>Solution</th>
<th>Rate of Diffusion (µS/cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% salt/water</td>
<td></td>
</tr>
<tr>
<td>5% salt/5% sugar</td>
<td></td>
</tr>
</tbody>
</table>

QUESTIONS

1. Which substance is leaving the dialysis bag? Which is entering the bag? Draw a picture of the movement of the solution molecules.
2. What quantitative data indicates that there is material leaving the dialysis bag? Does the concentration of the solution inside the bag affect the rate of diffusion of molecules?

3. In Part II of the lab, does the presence of a sugar molecule in solution outside the dialysis bag affect the rate of diffusion by the molecules inside the bag? Why is this?

4. Design an experiment that would quantitatively show that material is entering the dialysis bag.

5. In a different experiment, a solution of lactose (a double ring sugar) is placed inside the dialysis bag. How do you think the rate of diffusion of lactose will compare with the rate of sodium chloride diffusion?