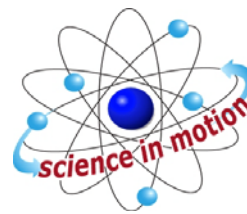


DIFFUSION THROUGH MEMBRANES



Westminster College

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 (H_2S 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 H_2S molecules than the room does and therefore the H_2S 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 H_2S molecules in the room, there will be net movement of the molecules. Eventually, dynamic equilibrium will be reached; the concentration of H_2S will be approximately equal throughout the room and no net movement of H_2S 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
 1%, 5%, and 10% salt water
 400 mL beaker
 ring stand and utility clamp
 distilled water & water bottle

dialysis tubing, 2.5 cm × 20 cm
 10 mL pipet & pipet pump
 plastic wrap
 plastic spoon
 5% sucrose (table sugar) solution
 tape & permanent marker
 disposable transfer pipet

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 $\mu\text{S}/\text{cm}$ 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 to return to the Meter mode screen.

d. A red box will now be in this window displaying a real time conductivity. Tap on the red box and select “Change Units” from the gray drop down menu. Select “mg/L” as the units for the experiment.

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. Select

. The screen should now look like the screen shown in Fig. 2.

Figure 1. Set-up of Diffusion Equipment

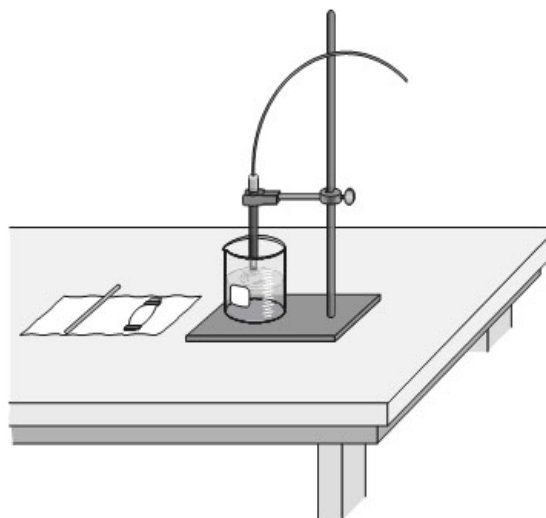
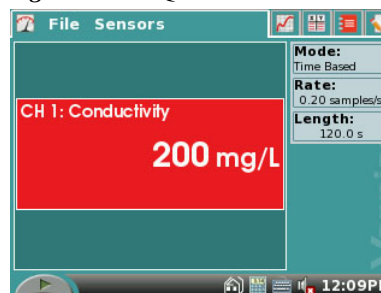


Figure 2. LabQuest Meter Screen

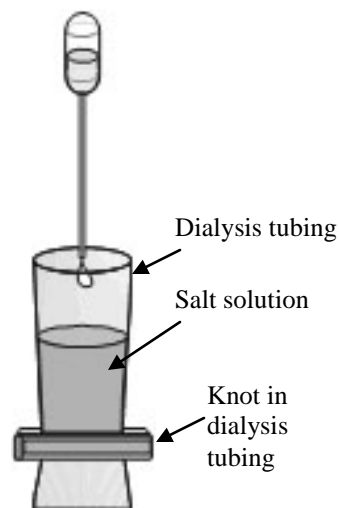


Part I. Concentration Gradients

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?

Figure 3. Dialysis tubing set-up



6. Prepare the dialysis tubing. Obtain a wet dialysis tube. Make a knot in one end of the tube about 1 cm from the end, as in *Figure 3*.


7. Place a 1% salt solution into a section of dialysis tubing (*Fig. 3*). To do this:

- Use the 10 mL pipet and pipet pump to obtain about 10 mL of the 1% salt solution.
- Use the pipet to transfer the 10 mL of the 1% salt water directly into the dialysis tube, as in *Figure 2*. **Note:** To open the tube, you may need to rub the tubing between your fingers.
- Tie off the top of the dialysis tube with a second knot. Try to keep any air bubbles to a minimum. The tube should be full, but not too tight, after it is tied.

8. Rinse the outside of the tubing with distilled 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.

- Secure the Conductivity Probe with the utility clamp in the water filled beaker as shown in *Figure 1*.
- Start the data collection by tapping the Start button .
- Place the 1% salt dialysis tube into the water. Be sure the tubing is completely submerged under the water. **Important:** Position the Conductivity Probe and dialysis tubing the same distance apart in each trial.
- Begin stirring the distilled water in the beaker with the plastic spoon. Stir the solution slowly and continuously throughout the two-minute data collection

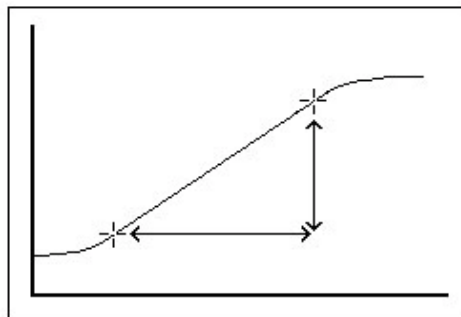
period. Data collection will stop automatically after 120 seconds. A graph of the 2 minute run will appear on the LabQuest screen.


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.


- While still in the Graph mode , choose Curve Fit from the Analyze menu.
- Select Linear for the Fit Equation. The linear-regression statistics for these two data columns are displayed for the equation in the form

$$y = mx + b$$
- Enter the absolute value of the slope, m , as the diffusion rate in mg/L/s for 1% salt solution in Table 2.
- Select

Figure 4. Highlight linear portion of curve



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 mg/L 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 mg/L 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 (mg/L/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

The College Board Advanced Placement Program. Biology Lab Manual for Students. Lab Two: Enzyme Catalysis. © 2001 by the College Examination Board. Pp. 1-4.

The College Board Advanced Placement Program. Biology Lab Manual for Teachers. Lab Two: Enzyme Catalysis. © 2001 by the College Examination Board. Pp. 1-4.

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(O₂), 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	
Part I	
Part II	

Part I:

Table 2: Rate of Salt Diffusion	
Salt Concentration (%)	Rate of Diffusion (mg/L/s)
1	
5	
10	

Part II:

Table 3: Conductivity Readings	
Solution	Conductivity (mg/L)
Distilled water	
5% sugar	

Table 4: Comparison of Diffusion	
Solution	Rate of Diffusion (mg/L/s)
5% salt/water	
5% salt/5% sugar	

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?