

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 is the process by which cells 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 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?

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 is capable of monitoring ions in solution. This probe however, will not measure the amount of electrically neutral molecules dissolved in water. Salts, such as sodium chloride, produce ions when they dissolve in water. If you place a salt solution in a container such as dialysis tubing, the salt can travel through the very small holes in the tubing. When dialysis tubing containing a solution of salt ions is placed into a beaker of water, the ions can diffuse out of the tubing and into the surrounding water. In this way, you will be able to measure the diffusion of salts in a solution of water and determine how concentration gradients and the presence of other particles affect the diffusion of the salt across a membrane.

OBJECTIVES

In this experiment, you will

- Use a computer and Conductivity Probe to measure the ionic concentration of various solutions.
- 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

computer
Vernier computer interface
LoggerPro
Vernier Conductivity Probe
three 18 × 150 mm test tubes with rack
1%, 5%, and 10% salt water
400 mL beaker

dialysis tubing, 2.5 cm × 12 cm
dropper pipet or Beral pipet
scissors
stirring rod
5% sucrose (table sugar) solution
dental floss or clamp
ring stand and utility clamp

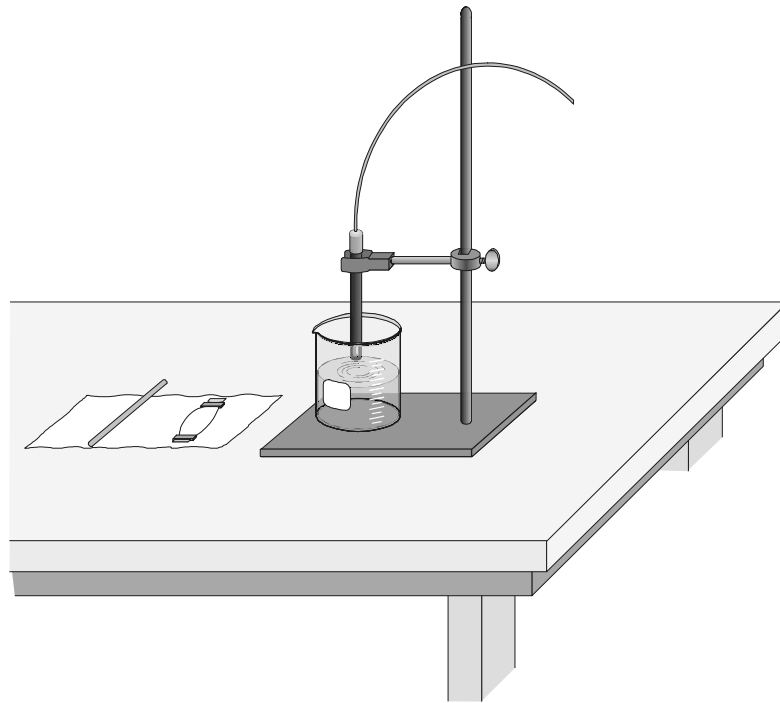


Figure 1

PROCEDURE

1. Connect the Conductivity Probe to the computer interface. Check to be sure the Conductivity Probe is set to the intermediate setting, $2000\ \mu\text{S}/\text{cm}$ (equivalent to a concentration of $1000\ \text{mg}/\text{L}$).
2. Prepare the computer for data collection by opening the file “04 Membrane Diffusion” from the *Biology with Vernier* folder of *LoggerPro*.

Part I Concentration gradients

3. Test whether different concentration gradients affect the rate of diffusion. You will place three solutions of differing salt concentrations (1%, 5%, and 10%) 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 water in the beaker will increase.
4. 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?
5. Prepare the dialysis tubing. Obtain a piece of wet dialysis tube and a dialysis tubing clamp or a short (approximately 10 cm) length of dental floss. Using the clamp or floss, tie one end of the tube closed about 1 cm from the end, as shown in Figure 2.

6. 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 a bit.
 - 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.

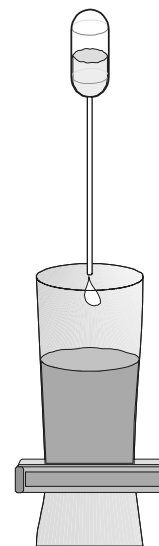



Figure 2

7. Place 300 mL of water into a 400 mL beaker. If the conductivity of the tap water is low (50 mg/L or less), use tap water to fill the beaker. Otherwise, use distilled water.
8. Position the Conductivity Probe into the water as shown in Figure 1. Place the dialysis tube into the water. Be sure the tubing is submerged completely under the water. **Important:** Be sure to position the Conductivity Probe and dialysis tubing the same distance apart in each trial.
9. After stirring the solution for 30 seconds, begin data collection by clicking . Stir the solution slowly and continuously throughout the two-minute data collection period. Data collection will automatically end after two minutes have passed.
10. Determine the rate of diffusion. To do this:
 - a. Move the mouse pointer to the point where the data values begin to increase. Hold down the mouse button. Drag the pointer to the end of the data and release the mouse button.
 - b. Click the Linear Fit button, , to perform a linear regression. A floating box will appear with the formula for a best fit line.
 - c. Record the slope of the line, m , as the rate of diffusion in Table 2.
 - d. Close the linear regression floating box.
11. 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.
12. Empty all of the liquid out of the dialysis tube. Squeeze the excess liquid out with your fingers.
13. Obtain 15 mL of a 5% salt solution in a test tube. Repeat Steps 6 – 12, substituting this 5% salt solution for the 1% solution.
14. Obtain 15 mL of a 10% salt solution in a test tube. Repeat Steps 6 – 12, substituting this 10% salt solution for the 1% solution.

Computer 4

15. Examine your data closely and make a conclusion. Record your conclusion in Table 1.

Part II Effect of other molecules

16. Measure the rate of diffusion of salt while it is in the presence of another, non-conducting solution. Since sugar does not form ions in solution, it should not conduct electricity. Sugar will be added to the water to determine whether it interferes with the diffusion of salt.

17. 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 diffusion rate of salt? Why?

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

18. Place some water in a clean 400 mL beaker.

19. Test the total dissolved solids concentration of the water by placing a clean Conductivity Probe into it. Record the total dissolved solid concentration value in Table 3. The total dissolved solids value should be displayed in the meter at the right of the screen.

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

21. Test the total dissolved solids concentration of the 5% sugar solution by placing a clean Conductivity Probe into it. Record the total dissolved solids value in Table 3. The total dissolved solids value should be displayed in the meter on the right of the screen.

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

22. Repeat Steps 6 – 12, with the following changes:

- Use 300 mL of sugar water in place of the water in Step 7.
- Substitute a 5% salt solution for the 1% solution.
- Record the rate of diffusion in Table 4.

23. Examine your data closely and make a conclusion. Record your conclusion in Table 1.

DATA

Table 1		
	Prediction	Conclusion
Part I		
Part II		

Part I

Table 2	
Salt concentration (%)	Rate of diffusion (mg/L/s)
1	
5	
10	

Part II

Table 3	
Solution	Concentration (mg/L)
Distilled water	
Sugar water	

Table 4: Summary of Data	
Solution	Rate of diffusion (mg/L/s)
5% salt	
5% salt / 5% sugar	

QUESTIONS

1. What conclusion can you draw from the data in Table 2?
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 $\mu\text{S/s}$ and the rate of the 10% solution was 5 $\mu\text{S/s}$, 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 ionic concentration of pure water with a sugar water solution. How do you account for this?
5. What conclusion can you draw from the data in Tables 3 and 4?

EXTENSIONS

1. Make a plot of the rate of diffusion *vs.* the salt concentration in the dialysis bag. 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.

Computer 4

4. Ectotherms are organisms whose body temperature varies with the surrounding environment. On the basis of on 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 a single celled organism were released by the organism into the pond, how would that affect the organism's ability to obtain oxygen as readily?

Vernier Lab Safety Instructions Disclaimer

THIS IS AN EVALUATION COPY OF THE VERNIER STUDENT LAB.

This copy does not include:

- **Safety information**
- **Essential instructor background information**
- **Directions for preparing solutions**
- **Important tips for successfully doing these labs**

The complete *Biology with Vernier* lab manual includes 31 labs and essential teacher information. The full lab book is available for purchase at:

<http://www.vernier.com/cmat/bwv.html>



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