

Biological Membranes

The primary objective of this experiment is to determine the stress that various factors, such as osmotic balance, detergents, and pH, have on biological membranes. Membranes within cells are composed mainly of lipids and proteins. They often serve to help maintain order within a cell by containing cellular materials.

One type of vacuole in the cells of plants, the *tonoplast*, is quite large and usually contains water. In beet plants, this membrane-bound vacuole also contains a water soluble red pigment, *betacyanin*, that gives the beet its characteristic color. Since the pigment is water soluble and not lipid soluble, it is contained in the vacuole when the cells are healthy. If the integrity of a membrane is disrupted, however, the contents of the vacuole will spill out into the surrounding environment and color it red. This usually means the cell is dead. If beet membranes are damaged, the red pigment will leak out into the surrounding environment. The intensity of color in the environment should be proportional to the amount of cellular damage.

You will test the effect of osmotic balance, detergents, and pH changes on biological membranes. The presence of certain salts is essential for most plant growth, but too much salt can kill plants. Even salts that are not transported across cell membranes can affect plants—by altering the osmotic balance. Osmosis is the movement of water across a semipermeable membrane from a region of low solute concentration to a region of higher solute concentration. It can greatly affect a cell's water content when the amount of water inside the cell is different than the amount outside the cell. You will test to see how this osmotic stress affects the cellular membrane integrity.

Detergents are designed to make lipids soluble in water. Since biological membranes are made of both lipids and water soluble materials, they are disrupted by detergents. You will design tests to determine the effect of this detergent on biological membranes.

The pH of an environment is critical for living things. If the environment is too acidic or too basic, organisms cannot survive. You will design tests to determine the effect of pH on biological membranes.

You will be using the Colorimeter or Spectrometer. In this device, blue light from the LED light source will pass through the solution and strike a photocell. The salt solutions used in this experiment are clear. If the beet pigment leaks into the solution, it will color the solution red. A higher concentration of colored solution absorbs more light and transmits less light than a solution of lower concentration. The computer-interfaced Colorimeter or Spectrometer monitors the light received by the photocell as either an *absorbance* or a *percentage transmittance* value. The *absorbance* of light will be used to monitor the extent of cellular membrane damage.



Figure 1

OBJECTIVES

In this experiment, you will

- Use a computer and a Colorimeter or Spectrometer to measure color changes due to disrupted cell membranes.
- Determine the effect of osmotic balance on biological membranes.
- Determine the effect of detergents on biological membranes.
- Determine the effect of pH on biological membranes.

MATERIALS

computer	Beral pipet(s) to transfer solutions
Vernier computer interface*	forceps
LoggerPro	knife
Colorimeter or Spectrometer	lab apron
100 mL beaker	pair of gloves
three 18 × 150 mm test tubes w/ rack	pH buffer solutions
microplate, 24-well	test tube rack
two 2 mL pipets	ruler (cm)
pipet pump or bulb	tap water
15% salt solution	timer or stopwatch
beet root	tissues (preferably lint free)
cotton swabs	toothpicks
detergent solution	

* Not necessary is using a Spectrometer

PROCEDURE

1. Obtain and wear goggles, an apron, and gloves.

Testing for the effect of osmotic stress

2. Obtain 10 mL of 15% salt solution and about 10 mL of tap water in labeled test tubes.
3. Prepare six salt solutions: 0%, 3%, 6%, 9%, 12%, and 15%. If your instructor has supplied graduated pipets with pipet pumps, add the *mL* of water specified in Table 1 to five of the six wells in the microwell plate. If your instructor has supplied Beral pipets, add the number of *drops* of water specified in Table 1 to each of four wells.
4. Use a different graduated pipet or Beral pipet to add 15% salt solution to five of the six wells in the microwell plate. See Table 1 to determine the volume of salt solution to add.
5. Clean the pipet used to transfer the salt solution.
6. Obtain a piece of beet from your instructor. Cut six squares, each 0.5 cm × 0.5 cm × 0.5 cm in size. They should fit into a microwell easily, without being wedged in. Be sure that:
 - there are no ragged edges.
 - no piece has any of the outer skin on it.
 - all of the pieces are the same size.
 - the pieces do not dry out.

7. Rinse the beet pieces twice using a small amount of water. Immediately drain off the water. This will wash off any pigment released during the cutting process.

Well number	H ₂ O		15% Salt		Concentration of salt (%)
	mL	drops	mL	drops	
1	2.4	60	0.0	0	0
2	1.9	48	0.5	12	3
3	1.4	32	1.0	24	6
4	1.0	24	1.4	32	9
5	0.5	12	1.9	48	12
6	0.0	0	2.4	60	15

8. Set the timer to 15 minutes and begin timing. Using forceps, add a piece of beet to each of the six well plates as shown in Figure 2. Stir the beet in the salt solution once every minute with a toothpick. Be careful not to puncture or damage the beet. While one team member is performing this step, another team member should proceed to Step 9.

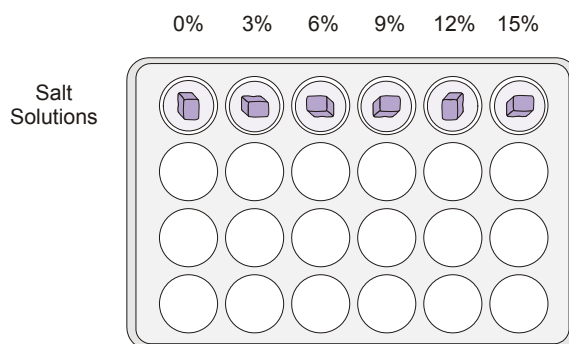



Figure 2

9. Prepare a *blank* by filling a cuvette 3/4 full with distilled water. To correctly use a cuvette, remember:
 - Wipe the outside of each cuvette with a lint-free tissue.
 - Handle cuvettes only by the top edge of the ribbed sides.
 - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
 - Always position the cuvette so the light passes through the clear sides.

Spectrometer Users Only (Colorimeter users proceed to the Colorimeter section)

10. Calibrate the Spectrometer.
 - a. Use a USB cable to connect the Spectrometer to your computer. Choose New from the File menu.
 - b. To calibrate the Spectrometer, place the blank cuvette into the cuvette slot of the Spectrometer, and choose Calibrate ► Spectrometer from the Experiment menu.

- c. The calibration dialog box will display the message: “Waiting 90 seconds for lamp to warm up.” After 90 seconds, the message will change to “Warmup complete.” Click Finish Calibration and then click .
11. Prepare the solutions for data collection.
 - a. After the 15-minute period is complete, remove the beet pieces from the wells. Remove them in the same order that they were placed into the wells. Discard the beet pieces and retain the colored solutions
 - b. Empty the blank. Transfer all of the 0% salt solution from Well 1 into the cuvette using a Beral pipet. Wipe the outside with a tissue and place it in the Spectrometer.
 12. Determine the optimum wavelength for creating the standard curve.
 - a. Click . A full spectrum graph of the solution will be displayed. Note that one area of the graph contains a peak absorbance. Click to complete the analysis.
 - b. To select a wavelength for analysis, click the Configure Spectrometer Data Collection icon, , in the toolbar.
 - c. Select Absorbance vs. Concentration (under the Collection Mode). The wavelength of maximum absorbance (λ max) will be selected. (It should be close to 540 nm.) Click . Leave the cuvette in the Spectrometer.
 - d. Proceed to Step 13.

Colorimeter Users Only

10. Connect the Colorimeter to the computer interface and prepare the computer for data collection by opening the file “09a Biological Membranes” from the *Biology with Vernier* folder of *LoggerPro*.
11. Calibrate the Colorimeter.
 - a. Open the Colorimeter lid.
 - b. Holding the blank cuvette by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.
 - c. Press the < or > button on the Colorimeter to select a wavelength of 470 nm (Blue) for this experiment. **Note:** If your Colorimeter has a knob to select the wavelength instead of arrow buttons, ask your instructor for calibration information.
 - d. Press the CAL button until the red LED begins to flash, then release. When the LED stops flashing, the calibration is complete.
12. Prepare the solutions and cuvette for data collection.
 - a. After the 10-minute period is complete, remove the beet pieces from the wells. Remove them in the same order that they were placed into the wells. Discard the beet pieces and retain the colored solutions.
 - b. Empty the water from the cuvette. Use a cotton swab to dry the cuvette after the water has been emptied from it.
 - c. Transfer all of the 0% salt solution from Well 1 into the cuvette using a Beral pipet. Wipe the outside with a tissue and place it in the Colorimeter. Close the lid. Proceed to Step 13.

Both Colorimeter and Spectrometer Users

13. You are now ready to collect absorbance data for the salt solutions.
 - a. Click to begin data collection.
 - b. Wait for the absorbance value displayed in the meter to stabilize.
 - c. Click , enter **0** in the edit box and then press ENTER. The data pair you just collected should now be plotted on the graph.
14. Collect the next data point.
 - a. Discard the cuvette contents into your waste beaker. Remove all of the solution from the cuvette. Use a cotton swab to dry the cuvette.
 - b. Fill the cuvette with the 3% salt solution from Well 2 using a Beral pipet. Wipe the outside with a tissue and place it in the device (close the lid if using a Colorimeter).
 - c. Wait for the absorbance value displayed in the meter to stabilize. Click , enter **3** in the edit box and then press ENTER.
15. Repeat Step 14 to save and plot absorbance and concentration values of the solutions in Wells 3, 4, 5, and 6. When you have finished with all of the salt solutions, click .
16. In Table 2, record the absorbance and concentration data pairs listed in the table.
17. Print a graph with the salt solutions plotted. Enter your name(s) and number of copies of the graph. Use your graph to answer the discussion questions at the end of this experiment.
18. Discard the solutions as directed by your teacher.

Testing for the effect of detergents

19. Predict what results you expect when cells are immersed in detergent.
20. Design a set of experiments that test the effect of a detergent on biological membranes.
21. In your lab book, describe how you would test the effect of detergent on biological membranes. Think about the equipment you will need in your experiment. You might want to use some of the materials listed in the materials table. Bring the procedure and the predictions for each of the six trials to class at the next meeting.

Day 2:

1. Have your instructor check the procedure you wrote. If it is approved, carry out the experiment. Make a data table that contains the data and describe the results and conclusions of your experiment.
2. If you are using a Colorimeter, prepare for data collection by opening the file “Exp 09b Biological Membranes” in the *Biology with Vernier* folder. Set the maximum x-axis value to the highest detergent concentration value in your test. If you are using a Spectrometer, set up the device as you did before.

Testing for the effect of pH changes

3. Predict what results you expect when the pH of the cells change from acidic to basic conditions.

Computer 9

4. In your lab book, describe how you would test the affect of pH changes on cell membranes. Bring the procedure and the predictions for each of the six trials to class at the next meeting.

Day 3:

1. Have your instructor check the procedure you wrote. If it is approved, carry out the experiment. Make a data table that contains the data and describe the results and conclusions of your experiment.
2. If you are using a Spectrometer, set up the device as you did before. If you are using a Colorimeter, prepare for data collection by opening the file “Exp 09c Biological Membranes” in the *Biology with Vernier* folder. **Note to Colorimeter users:** Although the Blue LED was used in the Colorimeter as a light source in the above experiments, use the Green LED setting when you measure pH effects. This is because at some pH values, the betacyanin pigment turns blue. When blue, the LED’s blue light is not absorbed by the pigment. **Important:** You must calibrate the Colorimeter as in Step 11, using the green setting in place of the blue setting.

DATA

Trial	Concentration of salt (%)	Absorbance
1	0	
2	3	
3	6	
4	9	
5	12	
6	15	

QUESTIONS

1. Which concentration of salt produced the most intensely red solution? The least intensely red solution?
2. Which salt concentration(s) had the least effect on the beet membrane? How did you arrive at this conclusion?
3. Did more damage occur at high or low salt concentrations? Explain why this might be so.
4. An effective way to kill a plant is to pour salt onto the ground where it grows. How might the salt prevent the plant’s growth? Is this consistent with your data?

Questions 5–8 refer to the experiment that tested the effect of detergents on membranes.

5. What effect did detergents have on cell membranes?

6. How did your answer in Question 5 compare to your prediction?
7. What assumptions did you make while designing your experiment that tested for the effect of detergents? How do you know they are valid assumptions to make?
8. How would you modify your experiment to either improve your results or to explore the validity of your assumptions?

Questions 9–12 refer to the experiment that tested the effect of pH on membranes.

9. What effect did changing the pH of the cell's environment have on cell membranes?
10. How did your answer in Question 9 compare to your prediction?
11. What assumptions did you make while designing your experiment in Day 2, testing for the effect of pH changes? How do you know they were valid assumptions to make?

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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