

Membrane Properties

Cells interact with their environment in a number of ways. Cells need to obtain nutrients from the environment, maintain water balance with their surroundings, and remove waste materials from the cell. As you have learned, the boundary between any cell and its environment is the plasma membrane, composed of a matrix of phospholipid molecules along with a number of different kinds of proteins. Membranes have different properties and a variety of functions, in large part determined by the specific proteins within the membrane. This exercise is designed to determine the stress that various factors, such as osmotic balance, detergents, and pH, have on biological membranes.

The central plant vacuole of plant cells contains water and a number of other molecules, including water-soluble pigments. Its membrane, the *tonoplast*, is normally poorly permeable to water. The central plant vacuole of beet root cells 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 remains in the vacuole when cells are healthy. If the integrity of the tonoplast and the plasma membrane is disrupted, however, the contents of the vacuole will spill out into the surrounding environment. The intensity of color in the environment is proportional to the amount of cellular membrane damage.

A colorimeter can be used to measure pigment intensity of the environmental solution. Blue light from the LED light source of the colorimeter will pass through the solution and strike a photocell. The test 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 pigment in the solution absorbs more light and transmits less light than a solution with less pigment. The computer-interfaced colorimeter monitors the light received by the photocell as either an *absorbance* or a *percentage transmittance* value. The output from our data recorder is the absorbance value. A higher absorbance value indicates more light has been absorbed by the environmental solution and thus, indicates more membrane damage.

You will have the opportunity to test the effect of alcohols, osmotic balance, detergents, and pH changes on biological membranes in this exercise.

- **Osmotic Balance**
Mineral ions and salts are essential for plant growth, but too much can kill plants (the "famous" over-fertilizing syndrome). Salt concentration affects osmotic balance. You will test to see how osmotic stress affects the cell membrane integrity.
- **Alcohols**
All alcohols are toxic to living organisms. One of the ways in which alcohols can cause damage is disruption of membrane structure. You will test the effect of methanol, ethanol and 1-propanol, three alcohols that differ only in the number of carbon and hydrogen atoms within the molecule, on membrane integrity.
- **Detergents**
Detergents are designed to make lipids soluble in water. The phospholipids in biological membranes are disrupted by detergents. You will design tests to determine the effect of detergent on biological membranes.
- **pH**
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.

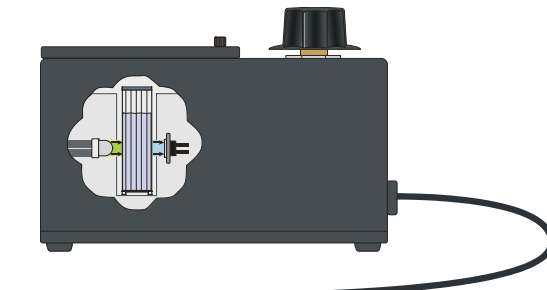
Materials Needed per Lab Group

Data Recorder (Computer)
Vernier LabPro and Power Supply
Vernier Colorimeter and Adapter
3 24-well microwell plates
Cuvettes
Fresh beet roots
15% salt (NaCl) solution
10 ml 1-propanol
10 ml ethanol
10 ml methanol
0.5% Detergent solution
Distilled water
Test tube rack
3 18-mm Test tubes
3 25 X 150 mm Test tubes
3 100-ml Beakers
Timer or stopwatch

pH Buffer solutions of:
pH 2
pH 4
pH 6
pH 7
pH 8
pH 10
pH 12
Cotton swabs
Several graduated pipettes or
medicine droppers
Forceps
Knife
Ruler
Kim wipes
Toothpicks
Graph paper

Preparing the Data Recorder and Colorimeter

1. Remove the data recorder and any LabPro interface materials from the data recorder cubicles. You may have to ask your instructor to unlock the cubicles.
2. Connect the colorimeter to the Vernier LabPro box and plug the Vernier LabPro box into the appropriate data recorder port. Be sure to do the connections before starting the computer. Start your data recorder (computer). Read the instructions on using the data recorder to locate the appropriate exercise.



Vernier Colorimeter

I. The Effect of Osmotic Balance on Membrane Integrity

You will prepare 6 salt (NaCl) solutions of differing concentrations (0%, 3%, 6%, 9%, 12% and 15%). A small piece of beet will be placed in each solution. After ten minutes, each salt solution will be transferred to a small, rectangular cuvette that is placed into the colorimeter. The amount of light that penetrates the solution and strikes the photocell is used to compute the absorbance of each solution. The absorbance is directly related to the amount of red pigment in the solution. By plotting the percent salt vs. the amount of pigment (that is, the absorbance), you can determine how salt concentration, and hence osmotic balance, affects membranes.

1. Open the "Biology with Vernier" folder on the data recorder and open "Exp 09A – Biological Membranes - Osmosis (Colorimeter)". If you are not familiar with the use of a trackpad to move the cursor on the screen, see the "How to use the Data Recorder" instructions handout provided with the laboratory exercises.

2. Be sure that you have a meter window with absorbance readings for the colorimeter. The vertical axis has absorbance scaled from 0 to 1.0. The horizontal axis has concentration scaled from 0 to 15%. If you do not have the meter window open, ask your instructor for assistance in configuring the computer.
3. Prepare a blank (needed to calibrate the colorimeter) by filling a cuvette 3/4 full with distilled water. Cuvettes must be used properly. To do so:
 - Clean the surface with a kimwipe to ensure that the cuvette is clean and dry.
 - Handle the cuvette only by the top edge of the ribbed sides.
 - Solutions in the cuvette should be free of air bubbles
 - Position the cuvette with a clear side facing the white reference mark at the right of the cuvette slot on the colorimeter
4. To calibrate your colorimeter, place the blank in the cuvette slot of the colorimeter.

If your colorimeter has an AUTO CAL button, set the wavelength of the colorimeter to 470nm (blue) and press the AUTO CAL button.

If your colorimeter does not have an AUTO CAL button, do the following:

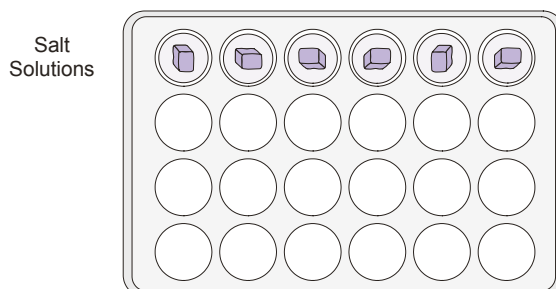
Choose "Calibrate" from the Experiment menu and then click .

- Turn the wavelength knob on the colorimeter to the "0% T" position.
- Type "0" in the box.
- When the displayed voltage for Input 1 stabilizes, click .
- Turn the knob of the colorimeter to the Blue LED position (470nm).
- Type "100" in the box.
- When the displayed voltage for Input 1 stabilizes, click , and then click .

5. Obtain 10 ml of 15% salt solution and about 10 ml of tap water. Place each into a labeled test tube.
6. Prepare six salt solutions: 0%, 3%, 6%, 9%, 12%, and 15%. Following the instructions in Table 1 below. Use a different pipette to add 15% salt solution to five of the six wells in the microwell plate.

Table 1			
Well number	H ₂ O	15% salt	Concentration of salt (%)
	drops	drops	
1	60	0	0
2	48	12	3
3	32	24	6
4	24	32	9
5	12	48	12
6	0	60	15

0% 3% 6% 9% 12% 15%



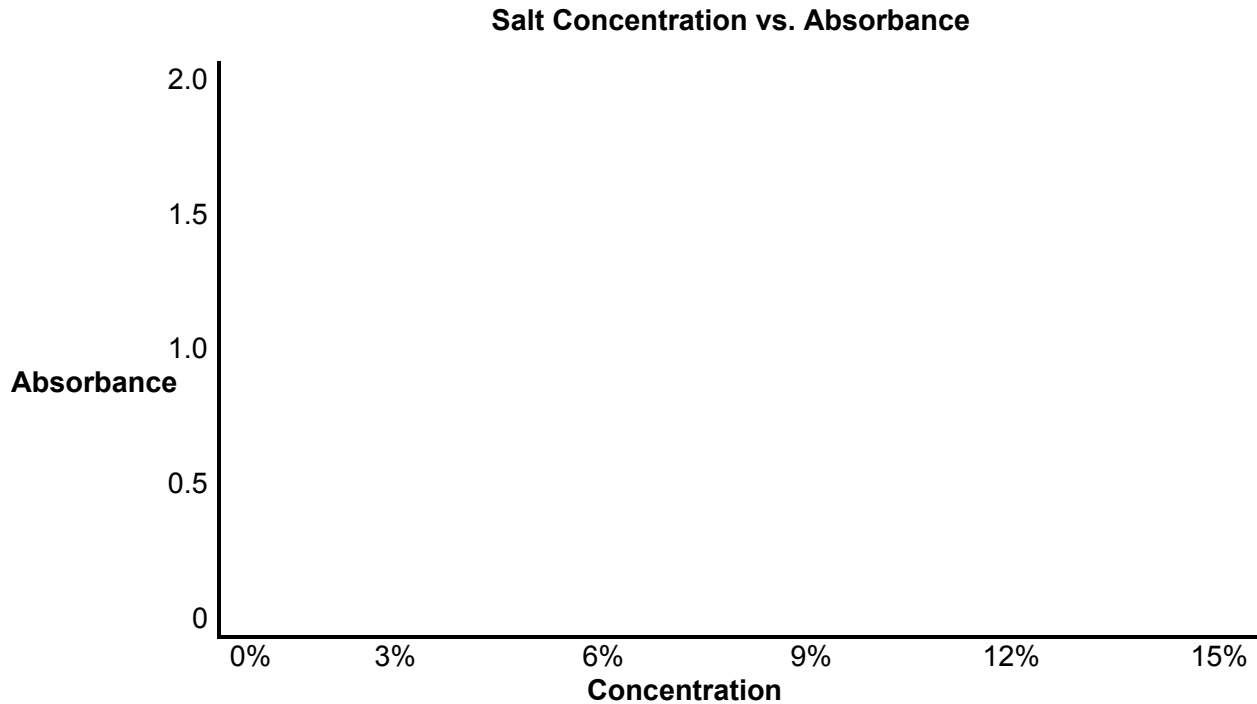
7. Obtain a piece of beet from your instructor. Cut six squares, each 0.5 cm X 0.5 cm X 0.5 cm in size. They should fit into a microwell easily, without being wedged in. While cutting the beet, be sure:
 - There are no ragged edges
 - No piece has any of the outer skin on it
 - All of the pieces are the same size
 - The pieces do not dry out
8. 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.
9. 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 below. Stir the beet in the salt solution once every minute with a toothpick. Be careful not to puncture or damage the beet.
10. After 15 minutes, remove the beet pieces from the wells. Remove them in the same order that they were placed in the well. Discard the beet pieces and retain the colored solutions.
11. You are now ready to collect absorbance data for the salt solutions.
 - Click .
 - Empty the water from the cuvette.
 - Use a cotton swab to dry the cuvette.
 - Transfer all of the 0% salt solution from Well 1 into the cuvette using a pipette.
 - Wipe the outside of the cuvette with a kimwipe and place it in the colorimeter.
 - Close the lid and wait for the absorbance value displayed in the Meter window to stabilize.
 - Click , enter "0" in the edit box and then press ENTER.
 - The data pair you just collected should now be plotted on the graph.
12. Discard the cuvette contents into your waste beaker
 - Use a cotton swab to dry the cuvette.
 - Fill the cuvette with the 3% salt solution from Well 2 using a pipette.
 - Wipe the outside of the cuvette with a kimwipe and place it in the colorimeter.
 - Close the lid and wait for the absorbance value displayed in the Meter window to stabilize.
 - Click , enter "3" in the edit box and then press ENTER.
 - The data pair you just collected should now be plotted on the graph.
13. Repeat step 12, 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 .
14. Record the absorbance and concentration data pairs listed in the "Microwell #" column in the Data Table below.

Data Table

Microwell #	Salt Concentration (%)	Absorbance
1	0	
2	3	
3	6	
4	9	
5	12	
6	15	

Discussion Questions

1. Using the data obtained for the salt solutions at the different concentrations, make a graph of concentration vs. absorbance.



2. Which concentration of salt produced the most intensely red solution? _____.
Which salt concentration produced the least intensely red solution? _____.
3. Which salt concentration(s) had the least effect on the beet membrane? _____.
How did you arrive at this conclusion?

Explain why this might be so.

5. 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 your response consistent with the data collected in this exercise?

II. Effect of Alcohol on Membrane Integrity

You will prepare five solutions of differing alcohol concentrations (0%, 10%, 20%, 30%, and 40%) for each of the three alcohols. A small piece of beet will be placed in each solution. After ten minutes, each alcohol solution will be transferred to a small, rectangular cuvette that is placed into the colorimeter. The amount of light that penetrates the solution and strikes the photocell is used to compute the absorbance of each solution. The absorbance is directly related to the amount of red pigment in the solution. By plotting the percent alcohol vs. the amount of pigment (that is, the absorbance), you can determine the amount of damage various alcohols cause to membranes.

Note: The alcohols used in this experiment are flammable and poisonous. Avoid inhaling vapors. Avoid contacting with skin or clothing. Be sure there are no open flames in the lab during this experiment. Gloves and goggles should be worn.

1. Open the "Biology with Vernier" folder on the data recorder and open "Exp 08 – The Effect of Alcohol on Biological Membranes (Colorimeter)".
2. Be sure that you have a meter window with absorbance readings for the colorimeter. The vertical axis has absorbance scaled from 0 to 1.0. The horizontal axis has concentration scaled from 0 to 70%. If you do not have the meter window open, ask your instructor for assistance in configuring the computer.
 - Clean the surface with a kimwipe to ensure that the cuvette is clean and dry.
 - Handle the cuvette only by the top edge of the ribbed sides.
 - Solutions in the cuvette should be free of air bubbles
 - Position the cuvette with a clear side facing the white reference mark at the right of the cuvette slot on the colorimeter

4. To calibrate your colorimeter, place the blank in the cuvette slot of the colorimeter.

If your colorimeter has an AUTO CAL button, set the wavelength of the colorimeter to 470nm (blue) and press the AUTO CAL button.

If your colorimeter does not have an AUTO CAL button, do the following:

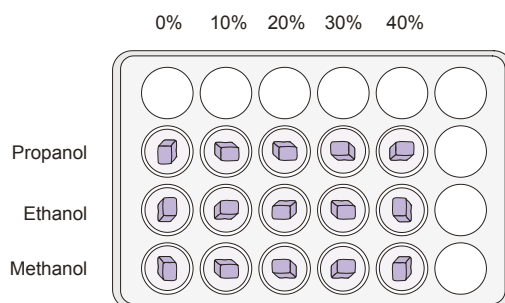
Choose "Calibrate" from the Experiment menu and then click .

- Turn the wavelength knob on the colorimeter to the "0% T" position.
- Type "0" in the box.
- When the displayed voltage for Input 1 stabilizes, click .
- Turn the knob of the colorimeter to the Blue LED position (470nm).
- Type "100" in the box.
- When the displayed voltage for Input 1 stabilizes, click , and then click .

5. Obtain a 24-well microplate and 3 test tubes. Label the test tubes M, E and P.
 - Pour 10 ml of methanol in the test tube labeled M.
 - Pour 10 ml of ethanol in the test tube labeled E.
 - Pour 10 ml of 1-propanol in the test tube labeled P.
 - Pour about 30 ml of tap water in a small beaker.
6. Prepare five **methanol** solutions (0%, 10%, 20%, 30% and 40%) according to the instructions in Table 1 below.
 - Use a pipette to add the number of **drops** of water as specified in Table 1 below to each of five wells in the microplate.
 - Use a second pipette to add the number of drops of **methanol** to each well in the microplate as specified in Table 1.

Well number	H ₂ O	Alcohol	Concentration of alcohol (%)
	drops	drops	
1	64	0	0
2	57	7	10
3	51	13	20
4	44	20	30
5	38	26	40

- Clean the pipette used to transfer the methanol. To do this, wipe the outside clean and empty it of liquid. Draw up a little **ethanol** into the pipette and use the liquid to rinse the inside of the pipette. Discard the ethanol.
- Prepare five **ethanol** solutions (0%, 10%, 20%, 30% and 40%) according to the instructions in Table 1 above.
 - Use a pipette to add the number of **drops** of water as specified in Table 1 to each of five wells in the microplate.
 - Use a second pipette to add the number of drops of **ethanol** to each well in the microplate as specified in Table 1.
- Clean the pipette used to transfer the ethanol. To do this, wipe the outside clean and empty it of liquid. Draw up a little **1-propanol** into the pipette and use the liquid to rinse the inside of the pipette. Discard the 1-propanol.
- Prepare five **1-propanol** solutions (0%, 10%, 20%, 30% and 40%) according to the instructions in Table 1 above.
 - Use a pipette to add the number of **drops** of water as specified in Table 1 to each of five wells in the microplate.
 - Use a second pipette to add the number of drops of **1-propanol** to each well in the microplate as specified in Table 1.
- Obtain a piece of beet. Cut 15 squares, each 0.5 cm X 0.5 cm X 0.5 cm in size. They should easily fit into a microwell of the microplate without being wedged in. While cutting the beet, be sure:
 - 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
- Rinse the beet pieces several times using a small amount of water. Immediately drain off the water. This will wash off any pigment released during the cutting process.
- Set the timer to 10 minutes and begin timing.
- Use forceps to place a piece of beet into each of 15 wells, as shown in the diagram below.



Stir the beet pieces in the alcohol solutions once every minute with a toothpick for 10 minutes. Be careful not to puncture or damage the beet. Do not let the toothpick get contaminated with the alcohol solutions.

15. After 10 minutes, remove the beet pieces from the wells. Remove them in the same order that they were placed in the well. Discard the beet pieces and retain the colored solutions.

16. You are now ready to collect absorbance data for the alcohol solutions.

- Click .
- Empty the water from the cuvette.
- Use a cotton swab to dry the cuvette.
- Transfer all of the 0% methanol solution from Well 1 into the cuvette using a pipette.
- Wipe the outside of the cuvette with a kimwipe and place it in the colorimeter.
- Close the lid and wait for the absorbance value displayed in the Meter window to stabilize.
- Click , enter "0" in the edit box and then press ENTER.
- The data pair you just collected should now be plotted on the graph.

17. Discard the cuvette contents into your waste beaker.

- Use a cotton swab to dry the cuvette.
- Fill the cuvette with the 10% methanol solution from Well 2 using a pipette.
- Wipe the outside of the cuvette with a kimwipe and place it in the colorimeter.
- Close the lid and wait for the absorbance value displayed in the Meter window to stabilize.
- Click , enter "10" in the edit box and then press ENTER.
- The data pair you just collected should now be plotted on the graph.

18. Repeat Step 19, using the solutions in wells 3, 4, and 5. After you click the button in the edit box, be sure you enter "20", "30", or "40" as appropriate for the solution being tested.

21. When you have finished with all of the methanol solutions click .

22. Record the absorbance and concentration data pairs listed in the "Microwell #" column in the Data Table below.

23. Store the data for the methanol solutions by choosing Store Latest Run from the Data menu.

24. Repeat Steps 18 through 23, measuring the five **ethanol** solutions.

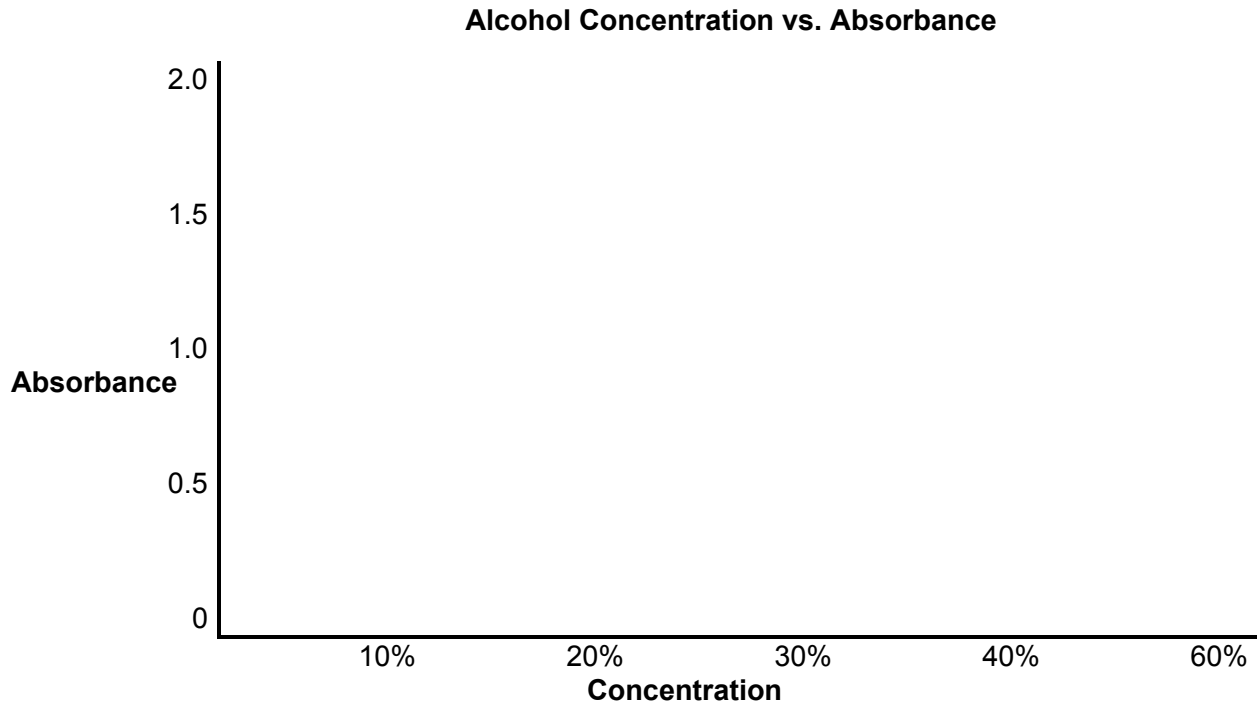
25 Repeat Steps 18 through 23, measuring the five **1-propanol** solutions.

Data Table

Microwell #	Concentration (%)	Absorbance		
		Methanol	Ethanol	1-Propanol
1	0			
2	10			
3	20			
4	30			
5	40			

Discussion Questions

1. Using the data obtained for the three alcohols at the different concentrations, make a graph of concentration vs. absorbance for the three alcohols.



2. At what percentage of alcohol is the cellular damage highest for methanol? _____
For ethanol? _____
For 1-propanol? _____
3. Which of the three alcohols seems to affect membranes the most? _____
Is there a relationship between the size of the alcohol molecule and the extent of membrane damage? Why might this be so?

III. **Effect of Detergents on Biological Membranes**

Based on the experiments you have completed with alcohols and salt solutions, design an experiment to test the effect of a detergent on biological membranes. Write out a list of materials and equipment needed and the procedure that you would use, along with your prediction. Have your instructor check your list of materials and your procedure. If time permits, you will do the exercise you have designed in this or the next laboratory period.

The data can be collected using the Exp 09B Colorimeter file in the "Biology with Vernier" folder on the data recorder. The vertical axis has Absorbance scaled from 0 to 1.0. The horizontal axis has Detergent concentration scaled from 0 to 100%. Set the maximum x-axis value to the highest detergent concentration value in your test.

After you have completed the exercise, make a data table and graph your results.

Discussion Questions

1. What effect did detergents have on cell membranes?
2. How did your answer compare to your prediction?
3. 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?
4. How would you modify your experiment to either improve your results or to explore the validity of your assumptions?

IV. Effect of pH on Biological Membranes

Based on the experiments you have completed, design an experiment to test the effect of pH on biological membranes. Write out a list of materials and equipment needed and the procedure that you would use, along with your prediction. Have your instructor check your list of materials and your procedure. If time permits, you will do the exercise you have designed in this or the next laboratory period.

The data can be collected using the Exp 09C Colorimeter file in the "Biology with Vernier" folder on the data recorder. The vertical axis has Absorbance scaled from 0 to 1.0. The horizontal axis has pH scaled from 0 to 12 pH units.

Although the Blue LED was used in the colorimeter as a light source in your previous experiments, use the Green LED setting when you measure pH effects. The betacyanin pigment turns blue at some pH values. When blue, the LED's blue light is not absorbed by the pigment.

Important: You must re-calibrate the colorimeter as in Step 4 of Procedure I (Effect of salt concentration) above, using the green setting in place of the blue setting.

After you have completed the exercise, make a data table and graph your results.

Discussion Questions

1. What effect did changing the pH of the cell's environment have on cell membranes?
2. How did your answer compare to your prediction?
3. What assumptions did you make while designing your experiment testing for the effect of pH changes? How do you know they were valid assumptions to make?
4. How would you modify your experiment to either improve your results or to explore the validity of your assumptions?

* Materials for this laboratory were modified from *Biology with Computers*, by Holman and Masterman © Vernier Software and Technology.