

The Effect of Alcohols on Biological Membranes

As you have learned, the boundary between any cell and its environment is the plasma membrane, composed of a matrix of phospholipid molecules with many 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 observe the effects of various alcohols on biological membranes.

The central plant vacuole of plant cells contains water and solutes, including water-soluble pigments. Its membrane, the *tonoplast*, is normally poorly permeable to water. The central plant vacuole of the root cells of beet 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 leave the vacuole and leach out into the surrounding environment. Membrane disruption generally occurs when the cell is dead.

You will test the effect of three different alcohols (methanol, ethanol, and 1-propanol) on beet vacuole membranes in this exercise.

- Ethanol, $\text{CH}_3\text{CH}_2\text{OH}$, an end product of alcohol fermentation, is found in alcoholic beverages.
- Methanol, CH_3OH , sometimes referred to as wood alcohol, can be fatal if consumed.
- Propanol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$, found in rubbing alcohol, can be fatal if consumed.

Methanol, ethanol and 1-propanol are very similar alcohols, differing only in the number of carbon and hydrogen atoms within the molecule. One possible reason why these alcohols are so toxic to living organisms is that they might damage membranes. If a beet cell's vacuole membrane (the tonoplast) is damaged, the red pigment, betacyanin, will leak out of the cell. The intensity of color in the environment should be proportional to the amount of cellular damage sustained by the beet.

To measure the color intensity, you will be using a colorimeter. Blue light from the LED light source of the colorimeter will pass through the solution and strike a photocell. The alcohol solutions used in this experiment are colorless. If the beet pigment leaks into the solution, the solution will turn beet-red. A higher concentration of pigment in the solution absorbs more light (and transmits less light) than a solution of lower pigment concentration. The computer-interfaced colorimeter can monitor the light received by the photocell as either an *absorbance* or a *percent transmittance* value.

Materials Needed

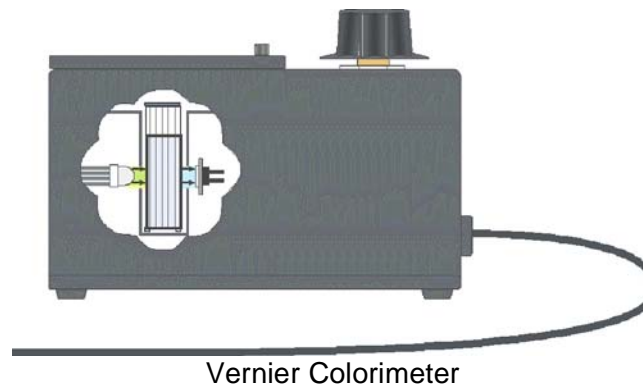
Data Recorder (Computer)	Cotton swabs
Vernier LabPro and Power Supply	Forceps
Vernier Colorimeter	Knife
Cuvettes	24-well microplate (microwell plate)
10 ml 1-propanol	Ruler
10 ml ethanol	Distilled water
10 ml methanol	Tap water
Fresh beet root	Timer or stopwatch
Test tube rack	Kim wipes
3 18-mm Test tubes	Toothpicks
100-ml Beaker	Graph paper
4 Graduated beral pipettes	

Note: The alcohols used in this experiment are flammable and poisonous. Be sure there are no open flames in the lab during this experiment. Gloves and goggles should be worn.

Procedure

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.

1. Remove the data recorder from the data recorder cubicles. You may have to ask your instructor to unlock the cubicles. Be sure that the data recorder is plugged into its power supply.
2. Connect the colorimeter to the Vernier interface box and plug the Vernier interface into its power supply if necessary and into the appropriate data recorder port. Start your data recorder. Be sure to do the connections before starting the computer. *For additional instructions about the data recorders see the handout, Using the Gateway Data Recorders.*



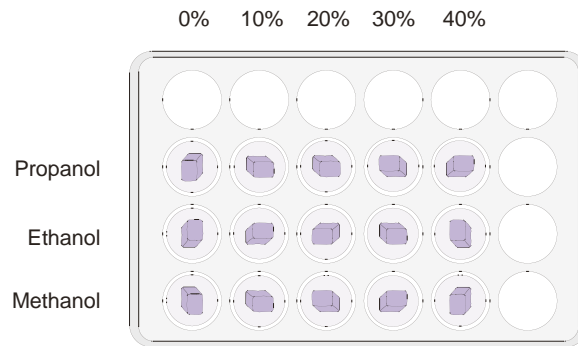
3. Double-click on the LoggerPro icon located on the computer desktop screen. Under the "file" menu, choose "Open", and open the Biology with Computers Folder. Select "Exp 08 – The Effect of Alcohol on Biological Membranes (Colorimeter)".
4. Be sure that you have a meter window with absorbance readings 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.
5. 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

6. To calibrate your colorimeter, place the blank in the cuvette slot of the colorimeter. 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 .
7. 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.
8. Prepare five **methanol** solutions (0%, 10%, 20%, 30% and 40%) according to the instructions in Table 1 below.
 - Use a beral pipet 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 Beral pipet to add the number of drops of **methanol** to each well in the microplate as specified in Table 1.

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

9. Clean the pipet 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.
10. Prepare five **ethanol** solutions (0%, 10%, 20%, 30% and 40%) according to the instructions in Table 1 above.
 - Use a beral pipet to add the number of **drops** of water as specified in Table 1 to each of five wells in the microplate.
 - Use a second Beral pipet to add the number of drops of **ethanol** to each well in the microplate as specified in Table 1.
11. Clean the pipet 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.
12. Prepare five **1-propanol** solutions (0%, 10%, 20%, 30% and 40%) according to the instructions in Table 1 above.
 - Use a beral pipet to add the number of **drops** of water as specified in Table 1 to each of five wells in the microplate.
 - Use a second Beral pipet to add the number of drops of **1-propanol** to each well in the microplate as specified in Table 1.

13. 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
14. 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.
15. Set the timer to 10 minutes and begin timing.
16. 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.

17. 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.
18. 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 Beral pipet.
 - 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 you just collected should now be plotted on the graph.

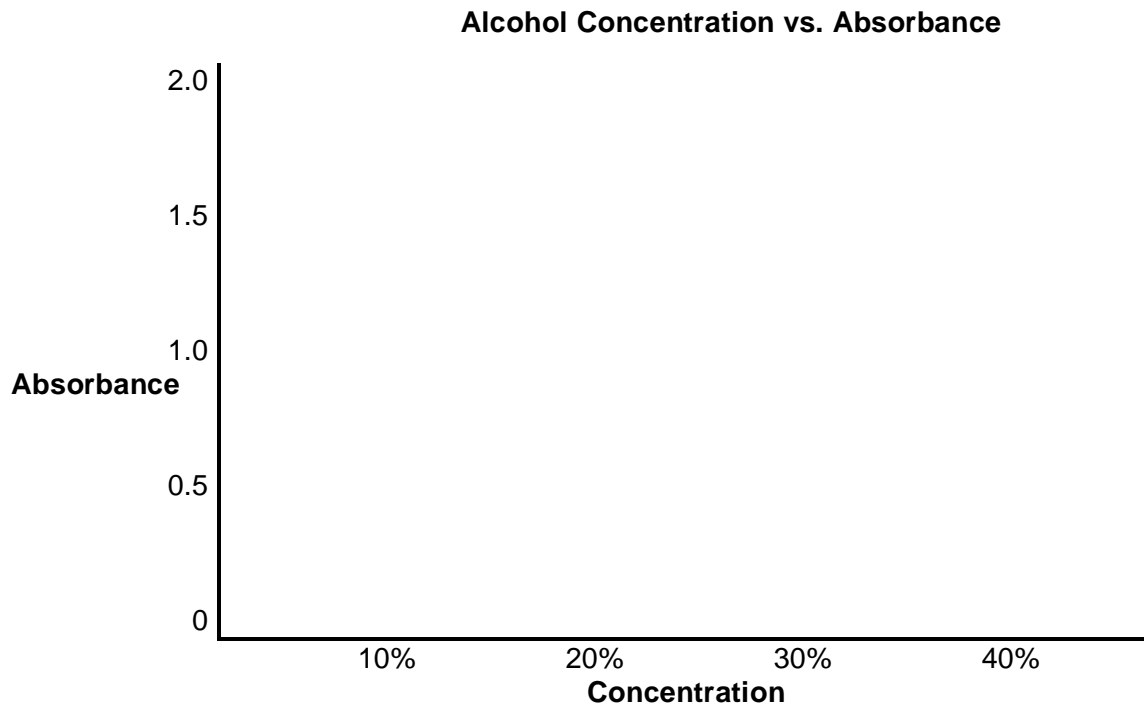
19. Discard the cuvette contents into your waste beaker.
 - Use a cotton swab to dry the cuvette.
 - Fill the cuvette with the 10% methanol solution from Well 2 using a Beral pipet.
 - Wipe the outside of the cuvette with a kimwipe and place it in the colorimeter.
 - Close the lid and wait for the absorbance 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.
20. 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?
Methanol _____
Ethanol _____
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?

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