The Effect of Alcohol on Biological Membranes

The primary objective of this experiment is to determine the stress that various alcohols have on biological membranes. Membranes within cells are composed mainly of lipids and proteins and often serve to help maintain order within a cell by containing cellular materials. Different membranes have a variety of specific functions.

One type of membrane-bound vacuole found in plant cells, 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 remains 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. This usually means the cell is dead.

In this experiment, you will test the effect of three different alcohols (methanol, ethanol, and 1-propanol) on membranes. Ethanol is found in alcoholic beverages. Methanol, sometimes referred to as wood alcohol, can cause blindness and death. Propanol is fatal if consumed. One possible reason why they are so dangerous to living organisms is that they might damage cellular membranes. Methanol, ethanol, and 1-propanol are very similar alcohols, differing by the number of carbon and hydrogen atoms within the molecule. Methanol, CH_3OH, is the smallest, ethanol, CH_3CH_2OH, is intermediate in size, and 1-propanol, $CH_3CH_2CH_2OH$, is the largest of the three molecules.

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 sustained by the beet.

To measure the color intensity, you will be using a Colorimeter. In this device, blue light from the LED light source will pass through the solution and strike a photocell. The alcohol 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 monitors the light received by the photocell as either an *absorbance* or a *percent transmittance* value.

You are to prepare five solutions of differing alcohol concentrations (0%, 10%, 20%, 30%, and 40%) for each of the three alcohols. A small piece of beet is placed in each solution. After ten minutes, each alcohol solution is 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 assess the amount of damage various alcohols cause to cell membranes.

OBJECTIVES

In this experiment, you will

- Use a Colorimeter to measure the color intensity of beet pigment in alcohol solutions.
- Test the effect of three different alcohols on membranes.
- Test the effect of different alcohol concentrations on membranes.

MATERIALS

computer
Vernier computer interface
Logger *Pro*
Vernier Colorimeter
four graduated Beral pipets
10 mL 1-propanol
10 mL ethanol
10 mL methanol
three 18 × 150 mm test tubes with rack
100 mL beaker
beet root

cotton swabs
forceps
knife
lab apron
microplate, 24-well
one pair gloves
ruler (cm)
tap water
timer or stopwatch
tissues (preferably lint-free)
toothpick

PROCEDURE

1. Obtain and wear goggles, an apron, and gloves. **CAUTION:** The compounds used in this experiment are flammable and poisonous. Avoid inhaling vapors. Avoid contacting them with your skin or clothing. Be sure there are no open flames in the lab during this experiment. Notify your teacher immediately if an accident occurs.

2. Obtain the following materials:

   a. Place about 10 mL of methanol in a medium sized test tube. Label this tube M.
   b. Place about 10 mL of ethanol in a medium sized test tube. Label this tube E.
   c. Place about 10 mL of 1-propanol in a medium sized test tube. Label this tube P.
   d. Place about 30 mL of tap water in a small beaker.

3. Prepare five methanol solutions (0%, 10%, 20%, 30% and 40%). Using Beral pipets, add the number of *drops* of water specified in Table 1 to each of five wells.

   Use a different Beral pipet to add alcohol to each of five wells in the microwell plate. See Table 1 to determine the number of drops of alcohol to add to each well.

Table 1			
Well number	H_2O	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

4. Clean the pipet used to transfer alcohol. 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.

5. Prepare five ethanol solutions. To do so, repeat Step 3, substituting ethanol for methanol. Place each solution in the second row of wells. See Figure 1.

6. Prepare five 1-propanol solutions. To do so, clean your pipette and repeat Step 3, substituting 1-propanol for methanol. Place each solution in the third row of wells. See Figure 1.

7. Now, obtain a piece of beet from your instructor. Cut 15 squares, each 0.5 cm x 0.5 cm x 0.5 cm in size. They should easily fit into a microwell 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 several times using a small amount of water. Immediately drain off the water. This will wash off any pigment released during the cutting process.
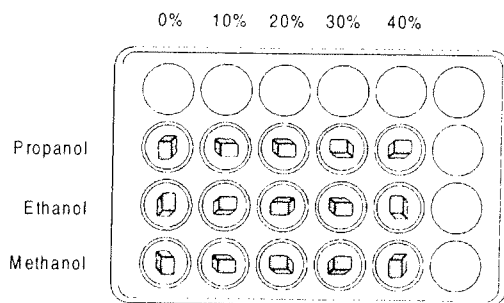

Figure 1

9. 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 Figure 1. Stir the beet in the alcohol solution once every minute with a toothpick. Be careful not to puncture or damage the beet.

10. Connect the Colorimeter to the computer interface. While one team member is performing Step 9, another team member should prepare the computer for data collection by opening the file "08 Alcohol and Membranes" from the *Biology with Computers* folder of Logger*Pro*.

11. You are now ready to calibrate the Colorimeter. Prepare a *blank* by filling a cuvette 3/4 full with distilled water. To correctly use a Colorimeter cuvette, remember:

   - All cuvettes should be wiped clean and dry on the outside with a tissue.
   - Handle cuvettes only by the top edge of the ribbed sides.
   - All solutions should be free of bubbles.
   - Always position the cuvette with its reference mark facing toward the white reference mark at the top of the cuvette slot on the Colorimeter.

12. Calibrate the Colorimeter.

   a. Open the Colorimeter lid.

   b. Holding the cuvette by the upper edges, place it in the cuvette slot of the Colorimeter. Close the lid.

   c. If your Colorimeter has a CAL button, Press the < or > button on the Colorimeter to select a wavelength of 470 nm (Blue) for this experiment. Press the CAL button until the red LED begins to flash. Then release the CAL button. When the LED stops flashing, the calibration is complete. Proceed directly to Step 13. If your Colorimeter does not have a CAL button, continue with this step to calibrate your Colorimeter.

   First Calibration Point

   d. Choose Calibrate ▶ CH1: Colorimeter (%T) from the Experiment menu and then click [Calibrate Now].

   e. Turn the wavelength knob on the Colorimeter to the "0% T" position.

   f. Type "0" in the edit box.

   g. When the displayed voltage reading for Reading 1 stabilizes, click [Keep].

   Second Calibration Point

   h. Turn the knob of the Colorimeter to the Green LED position (565 nm).

   i. Type "100" in the edit box.

   j. When the displayed voltage reading for Reading 2 stabilizes, click [Keep], then click [Done].

13. After 10 minutes, 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.

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

   a. Click [▶ Collect].

   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% methanol solution from Well 1 into the cuvette using a Beral pipet. Wipe the outside with a tissue and place it in the colorimeter. After closing the lid, wait for the absorbance value displayed in the meter to stabilize.

   d. Click [Keep], enter "0" in the edit box and then press ENTER. The data pair you just collected should now be plotted on the graph.

15. Discard the cuvette contents into your waste beaker. Remove all of the solution from the cuvette. 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 with a tissue and place it in the colorimeter. After closing the lid, wait for the absorbance value displayed in the meter to stabilize. Click [Keep], enter "10" in the edit box and then press ENTER.

16. Repeat Step 15, using the solutions in Wells 3, 4, and 5. When you have finished with all of the methanol solutions click [■ Stop].

17. In the data table, record the absorbance and concentration data pairs listed in the data table.

18. Store the data for the methanol solutions by choosing Store Latest Run from the Experiment menu.

19. Repeat Steps 14 through 18, measuring the five ethanol solutions.

20. Repeat Steps 14 through 17, measuring the five propanol solutions.

21. To print a graph of concentration *vs.* absorbance showing the data for all three alcohols:
    a. Label all three curves by choosing Text Annotation from the Insert menu, and typing "Ethanol" (or "Methanol", or "1-Propanol") in the edit box. Then drag each box to a position near its respective curve. Adjust the placement of the arrow head.
    b. Print a copy of the graph, with all three data sets displayed. Enter your name(s) and the number of copies of the graph you want.
    c. Use your graph to answer the discussion questions at the end of this experiment.

DATA

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

QUESTIONS

1. Which alcohol damaged the beet at the lowest concentrations? How did you determine this?

2. Which of the three alcohols seems to affect membranes the most? How did you come to this conclusion?

3. At what percentage of alcohol is the cellular damage highest for methanol? ethanol? 1-propanol?

CHALLENGE QUESTION

1. What is the relationship between the size of the alcohol molecule and the extent of membrane damage? Hypothesize why this might be so.