The purpose of this lab is to determine the stress that various alcohols have on biological membranes. Membranes of cells are composed mainly of lipids and proteins and serve to maintain cellular integrity 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 that made the vacuole 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 alcohols 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 or Spectrometer. In this device, light from the LED light source will pass through the solution and strike a photocell. The alcohol solutions used in this experiment are initially colorless. 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 device monitors the light received by the photocell as either an *absorbance* or a *percentage 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 cuvette that is placed into the Colorimeter or Spectrometer. 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

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

MATERIALS

LabQuest   cotton swabs
LabQuest App   forceps
Vernier Colorimeter or Spectrometer   knife
graph paper or Logger *Pro*   lab apron
two 2 mL pipets or 2 Beral pipets   microwell plate, 24-well
10 mL 1-propanol   toothpick
10 mL ethanol   one pair gloves
10 mL methanol   ruler (cm)
three 18 x 150 mm test tubes with rack   tap water
100 mL beaker   timer or stopwatch
pipet pump or pipet bulb   tissues (preferably lint-free)
beet root

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:

    - Place about 10 mL of methanol in a medium sized test tube. Label this tube *M*.
    - Place about 10 mL of ethanol in a medium sized test tube. Label this tube *E*.
    - Place about 10 mL of 1-propanol in a medium sized test tube. Label this tube *P*.
    - 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 in the microwell plate.

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

5. 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.

6. Prepare five ethanol solutions. To do so, repeat Steps 3 and 4, substituting ethanol for methanol. Place each solution in the second row of wells. See Figure 1.

7. Prepare five 1-propanol solutions. To do so, clean your pipette and repeat Steps 3 and 4, substituting 1-propanol for methanol. Place each solution in the third row of wells. See Figure 1.

8. 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.

9. 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.
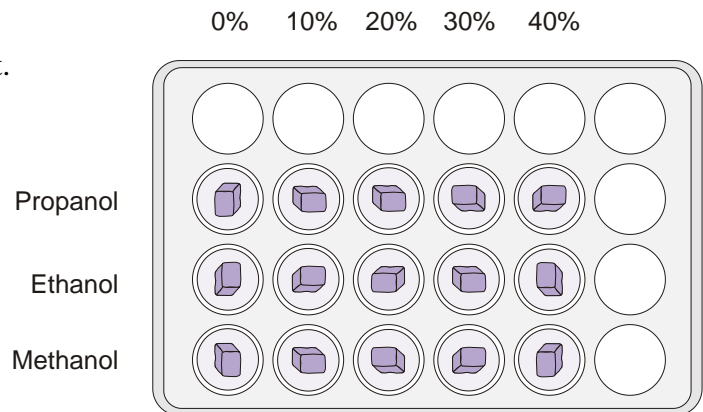

Figure 1

10. 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 2. Stir the beet in the alcohol solution once every minute with a toothpick. Be careful not to puncture or damage the beet. While one person is performing this step, another team member should proceed to Step 11.

11. Prepare a blank by filling an empty cuvette 3/4 full with distilled water. Seal the cuvette with a lid. 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.

12. Connect the Colorimeter to LabQuest and choose New from the File menu. If you have an older sensor that does not auto-ID, manually set up the sensor.

13. Calibrate the Colorimeter.

    a. Place the blank in the cuvette slot of the Colorimeter and close the lid.
    b. 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.
    c. Press the CAL button until the red LED begins to flash, then release. When the LED stops flashing, calibration is complete.

14. Set up the data-collection mode.

    a. On the Meter screen, tap Mode. Change the data-collection mode to Events with Entry.
    b. Enter the Entry Label (Conc) and Units (%).
    c. Select OK.

15. Prepare the solutions and cuvette for use.

    a. When the timer set to 10 minutes goes off, remove the beet pieces from the wells. Remove them in the same order in which they were placed in the well. Discard the beet pieces and retain the colored solutions.
    b. Empty the blank. 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. Close the lid. Proceed to Step 16.

16. Start data collection.

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

    a. Wait for the absorbance value to stabilize and tap Keep.
    b. Enter the concentration of alcohol from Well 1. Select OK. The absorbance and concentration values have now been saved.

18. 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. Transfer all of the methanol solution from Well 2 into the cuvette 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 to stabilize and tap Keep.
    d. Enter the concentration of alcohol from Well 2. Select OK. The absorbance and concentration values have now been saved.

19. Repeat Step 18 using the solutions in Wells 3, 4, and 5.

20. Stop data collection to view a graph of absorbance *vs.* concentration. To examine the data pairs on the displayed graph, select any data point.

21. Store the data from the first run by tapping the File Cabinet icon.

22. Discard the solution in the cuvette and transfer all of the ethanol solution from Well 1 into the cuvette using a Beral pipet. Wipe the outside with a tissue and place it in the device (close the lid if using a Colorimeter). Repeat Steps 16–21, substituting the ethanol solutions in place of the methanol.

23. Discard the solution in the cuvette and transfer all of the propanol solution from Well 1 into the cuvette using a Beral pipet. Wipe the outside with a tissue and place it in the device (close the lid if using a Colorimeter). Repeat Steps 16–20, substituting the propanol solutions in place of the methanol.

24. Graph all three runs of data on a single graph.

    a. Select Run 3, and select All Runs. All three runs will now be displayed on the same graph axes.

b. As you tap the data points, the absorbance and concentration values of each data point, for all three runs, are displayed to the right of the graph.

c. Record the absorbance values for all three runs in your data table (round to the nearest 0.001).

25. (Optional) Transfer your data to a computer for later printing per your teacher's instructions.

DATA

Table 2				
		Absorbance		
Trial	Concentration (%)	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?

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