

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, $\text{CH}_3\text{CH}_2\text{OH}$, is intermediate in size, and 1-propanol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$, 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, 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 device 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 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

- Use a Colorimeter or Spectrometer 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	cotton swabs
Vernier computer interface*	forceps
LoggerPro	knife
Colorimeter or Spectrometer	lab apron
four graduated Beral pipets	microplate, 24-well
10 mL 1-propanol	one pair gloves
10 mL ethanol	ruler (cm)
10 mL methanol	tap water
three 18 × 150 mm test tubes with rack	timer or stopwatch
100 mL beaker	tissues (preferably lint-free)
beet root	toothpick

* Not necessary if using a Spectrometer.

PROCEDURE

- 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.
- 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.
- 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.

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

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.

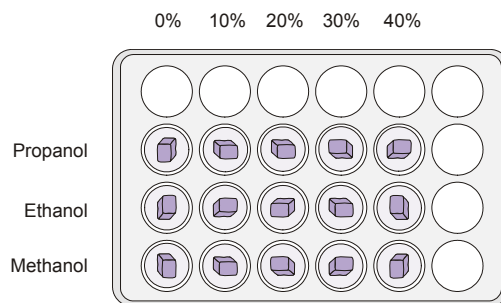



Figure 1

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. While one team member is performing this step, another team member should proceed to Step 10.
10. 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)

11. Calibrate the Spectrometer.
 - a. Use a USB cable to connect the Spectrometer to your computer. Choose New from the File menu.

Computer 8

- 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 .
12. Prepare the solutions 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. 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 Spectrometer.
13. 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 14.

Colorimeter Users Only

11. Connect the Colorimeter to the computer interface and prepare the computer for data collection by opening the file “08 Alcohol and Membranes” from the *Biology with Vernier* folder of *LoggerPro*.
12. 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.
13. 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% 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 14.

Both Colorimeter and Spectrometer Users

14. You are now ready to collect absorbance data for the alcohol solutions.
 - a. Click .
 - 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.
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 device (close the lid if using a Colorimeter). Wait for the absorbance value displayed in the meter to stabilize. Click , 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 .
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 14–18, measuring the five ethanol solutions.
20. Repeat Steps 14–17, 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.

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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