EXERCISE 5 - Lab Procedures

This lab requires a clock-watcher! Begin this lab exercise by setting up Parts II and III, and noting the time when you begin each activity. Then do Parts I, IV, and V while waiting for diffusion, osmosis, and dialysis to occur. The clock-watcher will be responsible for reminding the group when it is necessary to observe and measure the dye circles for Part II and when it is time to remove the dialysis bags for part III.

I. Observe the effects of molecular motion

Begin this activity after you have set up Parts II and III.

1. Place a drop of water on a clean microscope slide. Using a pair of forceps, break off a small piece of lichen (about the size of a match head) and place it in the drop of water. Use the blunt end of the forceps to thoroughly grind the lichen in the water into grayish green powder.

   NOTE: Lichens are organisms that superficially resemble plants but are not true plants. Instead, they are masses of interwoven cells from 2 different types of organisms – a fungus and an alga. The fungus is a non-photosynthetic organism and the alga is a photosynthetic protist. The fungal component and the algal component reproduce separately and remain distinct, yet interact as the lichen grows. Such a close, consistent co-existence is called **symbiosis** and the organisms involved are called **symbionts**.

2. Place a cover slip on the drop and view the ground-up lichen with the microscope. Find an area of the slide where the "lichen powder" is spread thinly, so there is plenty of light and you can see individual, separated grains of the powder.

3. Focus on low power first, and then switch to high power. Under high power, you should see tiny particles that are vibrating in place. The vibration is caused as water molecules on the slide move around and collide with the visible particles. This visible movement is called "Brownian motion" and is evidence for the kinetic theory of matter. Draw a diagram and describe your observations in your lab notebook.

II. Determine how molecular size and temperature affect the rate of diffusion

Begin this activity first.

1. Obtain the following materials:

2 room temperature agar plates, labeled A and B	Marking pen
1 refrigerated agar plate, labeled C	Ruler
Potassium permanganate solution	Micropipetters
Methylene blue solution	straw

2. Turn the agar plate labeled "A" upside down and make two small dots on the bottom of the plate with the marker. (Note: the bottom half of the agar plate is the side that contains the agar.) Each dot should be at least 2 cm from the edge of the plate and at least 3 cm away from the other dot on the bottom of the agar plate. Next to one dot, write "PP" (for potassium permanganate) and next to the other dot write "MB" (for methylene blue). Mark plates "B" and "C" the same way.

3. Remove the lid from agar plate "A." Use the straw to make a well in the agar at each dot that you have marked. To do this, fold the straw over at one end to make a tight seal and hold it with one hand. Then, pinch the straw with your fingers to expel some air from the straw. Then, bore a well in the agar with the opposite end of the straw by pushing it into the agar. Finally, release the fingers pinching the straw. This will create a small vacuum in the straw and will pull a plug of agar up into the straw. Discard this and repeat until your agar plates each have two wells.

4. Using a micropipettor, withdraw 25 µL of potassium permanganate solution and transfer it to the well that you have made directly above the correspondingly labeled dot. Do not let the well overflow. Use the same procedure to transfer 25 µL of methylene blue dye solution to your plate "A".

5. Repeat this for the potassium permanganate and methylene blue wells on plates "B" and "C." Work quickly with the "C" plate so that it remains cold.

6. Keep plates A and B at room temperature, and place plate C back in the refrigerator (or on ice if a refrigerator is unavailable). For the remainder of your lab period, measure the diameter of the dye circles every 15 minutes. Record your measurements in your lab notebook, using a table such as the one below:

Time (min)	**Potassium permanganate**			**Methylene blue**		
	Agar A	**Agar B**	**Agar C**	**Agar A**	**Agar B**	**Agar C**
0						
15						
30						
45						
60						
75						
90						

After you have prepared the agar plates with the dye solutions, begin Part III. Remember to observe your plates from this activity and record your measurements in your notebook every 15 minutes during the rest of the lab period.

III. Observe how water and solutes move across an artificial semipermeable membrane (dialysis tubing)

Begin this activity second.

1. Label four 200-300 mL beakers with the numbers 1-4. Fill each beaker with about 150 mL of the solutions listed in the table below.

   ⇒ **Always wear gloves when handling dialysis tubing!** Oils from your fingers will plug up the pores and ruin the tubing.

2. Cut 4 pieces of dialysis tubing if they haven't been provided precut for you. Each piece should be approximately 20 cm long.

3. Soak the dialysis tubing in a small beaker of distilled water for 1-2 minutes. Then use the following procedure to create 4 "dialysis bags" that you will fill with the solutions listed in the table below:

   a. Remove a piece of tubing from the distilled water and separate the sides to form a tube (similar to the way you separate the sides of a plastic produce bag at the grocery store.) Place a clip across the bottom of the tubing or carefully tie it closed. Take care to make a tight seal without tearing the bag.

   b. Fill the bag with approximately 10 mL of the appropriate solution (see the table below) using a 10 ml pipette. Be sure to use a clean pipette for each solution.

   c. Carefully squeeze air out of the bag and place a clip across the top of the bag, or tie it closed. **The closed bag should not be tightly filled**; leave enough room in the bag to allow it to swell as more water enters. Use a paper towel to carefully blot dry any spilled solution on the outside of the bag. Place the bag on a paper towel. You can write on the paper towel to identify the bags 1- 4.

Fill the beakers as follows:	Fill the bags as follows:
Beaker #1: distilled water	Bag #1: 1% starch solution
Beaker #2: distilled water	Bag #2: 20% glucose solution
Beaker #3: distilled water	Bag #3: 40% glucose solution
Beaker #4: 10% glucose solution	Bag #4: 10% glucose solution

4. After you have filled all of the bags and blotted them dry, weigh them, and record their “before dialysis” weights in a clearly labeled table in your lab notebook.

5. Place all the bags in their corresponding beakers at the same time and record the time.

6. When the bags have been in the beakers for at least 90 minutes (take more time if you have it), remove all of the bags from the beakers at the same time. **Do not discard the solutions in the beakers!** Rinse the bags with tap water, carefully blot them dry with paper towels, and place them on a clean paper towel, labeling the bags by writing on the paper towel.

7. Weigh the bags and enter the data in the table in your lab notebook in order to compare this weight with the weight of the dialysis bags before incubating them in the beakers of solutions.

8. Carry out a series of tests, using the 2 indicators you studied in part IV, to determine which solute(s) crossed the dialysis membrane and which solute(s) did not. **Record the results in your lab notebook and show them to your instructor before you discard the dialysis bags and the solutions in each beaker.**

IV. Determine which indicator to use to detect the presence of glucose or starch

In this part of the lab, you will determine how to use indicators to detect the presence of two substances: starch and a reducing sugar (such as glucose).

1. a. Label three test tubes B1, B2, and B3. To tube B1 add 1.0 ml dH_2O; to tube B2 add 1.0 ml 40% glucose solution; and to tube B3 add 1.0 ml 1% starch solution.

   b. Add 5 drops of the Benedict’s solution to each test tube. Note the appearance of each tube, and then place all three tubes in the boiling water bath for about 1 minute.

   c. Retrieve your test tubes from the boiling water bath. Describe the appearance of each tube after it was removed from the boiling water bath:

   Tube #1 ____ (dH_2O)

   Tube #2 ____ (glucose)

   Tube #3 ____ (starch)

   d. Benedict’s reagent can be used to detect the presence of which substance? _______________________

   e. The color for a positive test is _______________________

   f. The color for a negative test is ________________________

2. a. Label three test tubes L1, L2, and L3. To tube L1 add 1.0 ml dH_2O; to tube L2 add 1.0 ml 40% glucose solution; and to tube L3 add 1.0 ml 1% starch solution.

   b. Add 5 drops of Lugol's iodine to each test tube.

   c. Describe the appearance of each tube after the Lugol's iodine was added:

   Tube #1 ____________________________________ (dH_2O)

   Tube #2 ____________________________________ (glucose)

   Tube #3 ____________________________________ (starch)

   d. Lugol's iodine could be used to detect the presence of which substance? _________________________

   e. The color for a positive test is ______________________

   f. The color for a negative test is _______________________

V. Observe how osmosis affects living cells

1. Make a wet mount of an *Elodea* leaf.

You will do all of this section of the lab activity using <u>one</u> wet mount of an *Elodea* leaf.

Do not discard your wet mount until you have *completed* Part V!

2. Focus on the leaf using low power first, and then high power. While viewing the leaf under high power, draw a diagram of one cell labeling the chloroplasts, cytoplasm, plasma membrane, and cell wall.

3. Place a drop of the 10% NaCl solution on the right edge of the cover slip. **While looking through the microscope**, because you want to watch the cells' response *as it is occurring*, touch the corner of a Kimwipe to the left edge of the cover slip. This will draw the salt solution under the cover slip by capillary action.

4. In your lab notebook, describe what happens to the cells as the 10% salt solution flows over them. After waiting several minutes, draw another diagram of one cell, again labeling the chloroplasts, cytoplasm, plasma membrane, and cell wall. Briefly describe the difference between the 2 diagrams. **Ask your instructor to check both diagrams before proceeding to step 5.**

5. **Using the same slide,** repeat step #3 using distilled water instead of 10% NaCl. In your lab notebook, describe what happens to the cells as the distilled water flows over them.

Clean up

Dispose of your solutions in the proper waste containers.

Clean your glassware and place all equipment and solutions back where you found them. Leave your work area in the same order that you found it in.

All disposable glassware goes into the special glass disposal receptacle. Dialysis tubing may be disposed of in the waste paper baskets.

Wipe off your workspace with a damp paper towel.

Make sure everything that you have used is clean, put away, or discarded. Ask your instructor to check your work area before you leave.