Diffusion, Osmosis and Water Potential

1. Pre-lab Preparation

Complete the following reading before lab:

- Chapter 5 "Structure and Function Plasma Membranes" Biology2e, OpenStax College

2. Purpose and Objectives

The purpose of this lab is to investigate factors that affect the movement of materials into and out of cells.

Upon completion of this lab, you should be able to:

1. Understand how molecules move through a selectively permeable barrier.
2. Understand how osmosis affects living cells.
3. Accurately measure liquids using graduated cylinders and graduated pipettes.

Work in groups of two for this lab. In exercise 5 you will investigate the diffusion of molecules across a selectively permeable membrane. In exercise 6, you will investigate water potential in plant tissue.

3. Safety Guidelines

- Use all glassware with care. Be aware of the location of all lab equipment at all times.
- Wear safety goggles while working with solutions.
- Upon completion of the investigation, clean all equipment used, wipe down the lab table and wash hands before leaving the lab.

4. Measuring Volume and Mass

Measuring Volume of Liquids

Review, if needed, the metric units of volume.

Table 1: Metric Units of Volume

Milliliter (mL)	1/1000th liter
Microliter (µL)	1/1,000,000th liter

This week we will review measuring liquid volumes using graduated cylinders and graduated pipettes. Table 2 should be used as a guide to decide what type of equipment to use when measuring various volumes of liquid. It is generally preferable to use the device which has the smallest possible maximum volume.

Table 2: Guide for Choosing the Appropriate Device for Measuring Liquid Volume

Volume	Device
≤ 1 mL	Micropipetman
1 to 10 mL	Graduated Pipette
> 10 mL	Graduated Cylinder (smallest size possible)

Graduated pipettes

Graduated pipettes are generally used to measure liquid volumes between one and ten mL. As mentioned above, you should choose the smallest pipette possible for measuring the volume you want. You will commonly see pipettes that hold a maximum volume of 1 mL, 5 mL, or 10 mL. These are used with a pipette pump which generates enough suction to fill the pipette with liquid. **We will never pipette liquids by mouth in the Biology lab.**

Before you begin measuring liquids with a pipette it is important to check the gradations on the side. Some pipettes are numbered from bottom to top; others are numbered from top to bottom. Some pipettes have a zero mark above the end of the pipette and a "dead volume" which is not dispensed, near the tip. Other pipettes (referred to as serological or blow-out pipettes) do not have a dead volume and should be emptied completely to dispense the correct volume. Also, check the units on the pipette to determine what volume is represented by each gradation mark as they can vary as well.

To begin, attach the pipette firmly to the pipette pump so that liquid will not leak out when the pipette is full. **Do not touch the tip of the pipette as this will contaminate the pipette and the liquid into which you place it.** Place the tip of the pipette into the liquid that you want to measure. Dial up the pipette pump until the pipette is filled with the appropriate amount of liquid. **Be careful not to over fill the pipette as this will contaminate the pump.** Check the volume by looking at the pipette from the side at eye level. The bottom of the **meniscus*** should touch the gradation line for the volume you want to measure. Next, move the pipette to the container into which you want to dispense the liquid. Release the liquid by pressing the release bar or dialing down the pump. **Do not return any unused volume to the stock container.**

*The surface of some liquids (including those containing primarily water) will have a noticeable concave curve when the liquid is placed in a narrow container. This curve is called the **meniscus**. It results from the ability of the liquid to adhere to the sides of the container.

Graduated Cylinder

Most of you should be familiar with use of the graduated cylinder. Although beakers and flasks commonly have volume markings, these are not usually very accurate and the graduated cylinder is used to measure liquid volumes larger than about 10 mL. As with pipettes, they come in various sizes. Fifty mL, 100 mL, 250 mL, 500 mL, and 1 L cylinders are common. Smaller

cylinders are also sometimes used and you should always use the smallest possible cylinder to measure the volume needed.

As with pipettes, check the gradations on the side of the cylinder before you begin and calculate the volume of each gradation as cylinders vary. Next, pour the liquid into the cylinder, being careful not to overshoot the desired mark. Check the volume by looking at the cylinder from the side at eye level. The bottom of the **meniscus** should just touch the gradation line for the volume you want to measure. Next, carefully pour the liquid into the desired container. **Do not return any unused volume to the stock container.**

Use of the Top-loading Balance to Measure Mass

Measuring the mass of objects is also a common task in the Biology laboratory. The base unit of mass in the metric system is the gram (g). The top loading balances used in Biology lab have a precision of about 0.1 g. To measure the mass of an object or material proceed as follows:

Turn on the balance and let it warm up for a few seconds. The display should read 0.000 g. Make sure the display is set to grams.

Place an empty weigh boat or other container on the balance pan. Press the "zero" or "tare" button to set the weight of this container to zero.

Add the material that you want to measure to the weigh boat. For some materials the instructor may request that you first remove the weigh boat from the balance pan to avoid spilling on the balance. If you accidentally add too much material to the weigh boat **do not return the excess to the stock container. You should also be careful to avoid cross contamination of weigh boats and spatulas.**

Let the digital display stabilize and read the mass indicated.

Remove all material from the balance and clean up any spills.

Procedure

1. Determine the volume of liquid in graduated cylinders A, B, and C on the lab bench. Record these numbers in your lab notebook.

2. Obtain a 10 mL pipette, pipette pump, beaker of deionized water **(do not pipette out of the squeeze bottles)** and weigh boat.

3. Place the weigh boat on the balance pan and zero the balance. Lab partners should take turns making the measurements below.

4. Pipette 7 mL of water into the weigh boat. Record the mass of the water in your lab notebook.

5. Measure and record the mass for 2.5 ml water using a 5 ml pipette. Record in your lab notebook.

5. Diffusion Across a Selectively Permeable Membrane

Diffusion is the passive random movement of molecules from an area of high concentration to an area of low concentration. In other words, molecules are described as moving **down a concentration gradient**. If you have ever been in an elevator with an individual wearing perfume or cologne, you know that it does not take long for the scent to be experienced by all riding the same elevator. The perfume diffuses from where it is concentrated (on the wearer) throughout the air of the elevator (air currents also help move the perfume molecules). The movement of molecules continues until a **dynamic equilibrium** is reached, the point at which there is no **net** movement of molecules and the concentration of perfume molecules is the same throughout the space.

Diffusion involves the movement of molecules that are parts of **solutions**, mixtures of substances having a uniform composition. Solutions consist of two components: the **solvent**, which is the dissolving medium of a solution and usually present in the greatest amount, and the

solute or substance dissolved in the solvent. Solute concentration, temperature, and pressure will affect the direction in which a molecule will move. The rate at which molecules move is determined principally by their molecular weight and the difference in concentration between two areas (i.e., how steep the concentration gradient is). The ability of molecules to move from areas of high to low concentration is a form of chemical energy (**chemical potential**) that cells use to do work. In general, the greater the difference in concentration between two areas the greater the chemical potential energy. There would be no life if cells could not establish, maintain, and regulate differences in chemical concentration between themselves and their environment.

Membranes surround cells and many organelles. The ability to maintain distinct internal compartments allows cells to carry out a large number of chemical reactions simultaneously. Membranes are selectively and differentially permeable, allowing the cell to regulate the contents of the cytoplasm and organelle lumens. The movement of solutes across a selectively permeable membrane is called **dialysis**.

In this exercise, you will use a piece of dialysis tubing to make a model cell membrane. The tubing is made of cellulose and has millions of tiny pores through which substances, depending on their size, can move. Tubing can be purchased in a variety of pore sizes.

You will be placing a mixture of glucose and starch solutions into a bag made from dialysis tubing. The bag will then be placed into a beaker containing diluted Lugol's solution. Lugol's is a mixture of iodine (I_2), potassium iodide (KI) and water. The question being addressed is: Will any of the molecules in the solutions move through the dialysis tubing?

Procedure

1. Formulate a hypothesis describing which molecules (glucose, starch, iodine), if any, will be able to pass through the dialysis tubing. Make a prediction based on your hypothesis. The prediction should tell what the color of the solutions inside and outside the bag will be at the conclusion of the experiment if your hypothesis is supported. Also, what will be the result of the glucose tests? Record these in your lab book.

2. Fill a cup 2/3 full with deionized water. Wearing goggles and gloves, add 10 drops of Lugol's solution to the water in the cup. Be sure to note the color of this solution in your lab manual (initial color). Read the directions on the Diastix bag for proper use of the strips. Test the solution in the beaker for the presence of glucose using Diastix and record the results.

3. Wearing gloves, tie a knot in one end of a piece of dialysis tubing.

4. Place 5 mL of 15% glucose solution and 5 mL of 0.6% starch solution inside the bag. Gently mix the contents of the bag. Be sure to note the color of the solution inside the bag.

5. Gently rinse the outside of the bag with deionized water.

6. Place the dialysis bag into the cup so that the untied end of the bag hangs over the side. Secure the exposed end of the bag using the clip provided. If the bag is too full, remove some of the liquid and rinse the outside of the bag again prior to securing it with the clip.

7. Allow the setup to stand for one hour. Record any color changes in your lab book.

8. Test the solution in the beaker with a Diastix strip. Record your results.

9. Empty the bag and cup contents in the waste container provided.

6. Osmosis and Water Potential

Osmosis is the movement of water across a selectively permeable membrane. Most solutes cannot cross cell membranes by passive diffusion, but water can. Water will move from an area of high concentration to an area of low concentration. In this case, the concentration of water molecules is related to how much solute is dissolved in the water - the higher the solute concentration, the lower the concentration of water molecules.

If a cell finds itself in a solution that has a higher concentration of solutes than that found inside the cell, the solution is described as being **hypertonic**. When a cell is in a hypertonic environment water will move out of the cell (down its concentration gradient) into the

surrounding solution. If the cell finds itself in a solution that has a lower solute concentration relative to that found inside the cell (a **hypotonic** solution), water will move into the cell. In a solution that has the same solute concentration as the inside of the cell, an **isotonic** solution, no net movement of water will occur. Like all molecules, the diffusion of water will continue until equilibrium is reached.

Animal cells are normally found in an isotonic environment. Some animal cells are relatively impermeable to water and water moves into or out of these cells very slowly. Others (including red blood cells and some kidney cells) have membrane channels called aquaporins that allow for large amounts of water to cross their membranes. When removed from the body and placed in non-isotonic solutions in the laboratory these cells will be greatly affected. When placed in a hypotonic environment such as plain water, so much water may enter the cell that the pressure inside the cell increases to the point where it exceeds the resistance of the cell membrane and **cell lysis** occurs (the cell bursts open). When placed in a hypertonic environment such as salty water the cells may lose so much water that they will shrivel.

Movement of Water in Plants

In plants water is lost through a process known as **transpiration**, which occurs in the leaves. Water lost in this way is replaced by water that is taken up by the roots. Many plant cells are quite permeable to water and have been found to have aquaporin channels similar to those found in some animal cells.

Unlike animal cells, plant cells are protected from lysis in a hypotonic environment by the presence of a cell wall. As in animal cells water will move into a plant cell due to a difference in solute concentration inside the cell as compared to outside the cell. The force that causes this to happen is called **solute potential**. As water enters a cell placed in a hypotonic environment physical pressure builds up in the cell because the cell wall prevents the cell from expanding to accommodate the inflow of water. When the physical pressure is high enough water inflow will stop. This physical pressure is called **pressure potential**. High pressure potential may stop inflow of water into the cell even if the concentration of solutes is lower outside the cell than inside.

The movement of water into or out of cells is influenced by both pressure potential and solute potential. A measurement known as **water potential,** abbreviated by the Greek symbol **Ψ** takes into account both physical pressure and solute pressure. Water potential is calculated by adding together the pressure potential and solute potential.

Ψ	=	Ψ_p	+	Ψ_s
Water potential	=	pressure potential	+	solute potential

If the water potential (pressure potential plus solute potential) outside a cell is greater than inside the cell, water will move into the cell. As discussed above, in plants, the cell wall limits how much water can actually enter the cell. Plant cells placed in a hypotonic (high water potential) environment become **turgid** as the pressure potential rises. When the contents of a plant cell press the plasma membrane against the cell wall, the pressure generated is called **turgor pressure**. High turgor pressure within plant cells helps to keep the plant erect.

In this experiment, we will use the movement of water into and out of cells to determine and compare the water potential found in the roots or other parts of different plants.

Procedure

1. Each lab group is supplied with a set of plant sections taken from a different plant. Formulate a hypothesis describing which plant has the lowest (most negative) water potential (depends mostly on concentration of solutes; a more negative water potential would result from a higher concentration of solutes in the plant cells). Record this in your lab book. Based on your hypothesis, write a prediction describing how the results will support your hypothesis (hint: this has to do with the concentration of sucrose at which the vegetable with the most negative water potential will reach equilibrium and then begin to lose water).

2. At your bench you will find a set of containers, each of which holds 4 or more sections taken from a plant. All sections have been soaking overnight in 50 mL of either deionized water or

one of the following sucrose solutions: 0.2 M, 0.4 M, 0.6 M, or 0.8 M sucrose. On the board you will find a table that lists the mass of each group of 4 sections before being placed in solution. Record this information in a table in your lab book as **initial mass**.

3. Record the temperature of one of the solutions.

4. Wearing gloves, remove all of the plant sections from one of the containers. Gently blot them dry using a paper towel. Determine the collective mass of the 4 sections (put them all on the scale together). Record this information in your lab book as final mass. Return the plant sections to their container. **Be careful to return plant sections to their container before opening another container.** It is important that you do not mix up the samples.

5. Repeat this procedure for the remaining sections at your bench.

6. In your lab notebook, make note of any changes in the appearance or turgidity of the sections in each of the solutions.

7. Input your data on the excel sheet on the classroom computer. Calculate the percent change in mass in the different solutions for your vegetable using excel. Percent change is (final mass – initial mass)/initial mass. Record the percent change values for each of the vegetables used by your lab class in a second table.

8. Graph the percent change in mass of each vegetable (y-axis) against sucrose concentration (x-axis). Data for all the vegetables can be plotted on the same graph. Connect the points on the graph; do not draw a best-fit line. The point at which the line crosses the x-axis represents the molar concentration (C) of solutes at which there is no net movement of water across cell membranes in the plant sections. Record this number **for each of the sample sets weighed by the class.**

Calculating Water Potential

1. The solute potential for a solution can be calculated using the following formula:

$$\Psi_s = -iCRT$$

i = ionization constant, which for sucrose is 1.0 because sucrose does not ionize in water

C = molar concentration of solutes (determined from the graph)

R = pressure constant = 0.0831 liter bars/mole °K

T = temperature in °K = 273 + °C of the solution

2. Given that the pressure potential (Ψ_p) of the sucrose solution is 0, the water potential of the plant cells tested will be equal to the solute potential (Ψ_s) of the solution at which net water movement stops.

3. Calculate the water potential for each vegetable used by the class and record the values in your lab book. **Show your calculations**.

7. Conclusion and Summary

1. In the diffusion experiment (section 5), what molecule(s) moved? Did molecules move from higher to lower concentration or from lower to higher concentration?

2. Did the results for the diffusion experiment support your hypothesis? Why or why not?

3. What factor determined whether molecules could cross the selectively permeable barrier (dialysis tubing)?

4. In the water potential experiment (section 6) what molecule(s) moved across the plant cell membranes? For the plant sample that your group tested, what direction did this molecule(s) move at 0 M sucrose? At 0.8 M sucrose?

5. Which plant sample had the highest (most negative) water potential? Did this result support your hypothesis?

6. Were any of the results obtained in either of the experiments unexpected? How might you account for these unexpected results?

7. What are possible sources of error that could have affected your results? Explain.

8. Were there any limitations in the designs of the experiments that might have affected your results (i.e., is there anything about how the experiment was set up or conducted that limits what information can be obtained)? Explain.

9. Describe three ways the investigation could be extended. What kind of information would you hope to gain by extending the investigation further?

NOTES: