

Name _____ Instructor _____ Lab Section _____

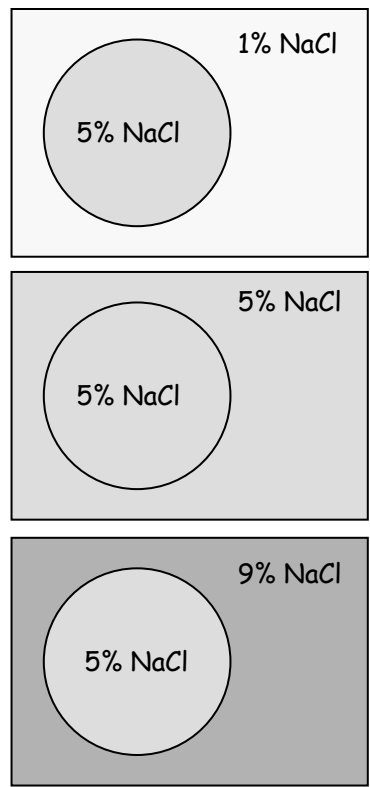
<p>Objectives: To gain an understanding of:</p> <ul style="list-style-type: none"> • The basic principles of osmosis and diffusion • Brownian motion • The effects of osmosis on plants and animals 	<p>Background material may be found in</p> <ul style="list-style-type: none"> • Chapter 5.3 - 5.5 • Chapter 25.4 <p><i>Biology: Concepts & Connections, 8th ed.</i></p>
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Physical, chemical and biological laws govern the exchange of substances between cells and their environment. In this lab, you will specifically deal with the properties of **cell membranes** and the **diffusion** of substances into and out of the cells. To perform their functions, cells must maintain a steady state in the midst of an ever-changing environment. This constancy is maintained by the regulation of the movement of materials into and out of the cell. To achieve this control, cells are bounded by a delicate membrane that can distinguish between different substances, slowing down the movement of some while allowing others to pass through. Because not all substances penetrate the membrane equally well, the membrane is said to be **selectively permeable**.

One way chemicals move into and out of cells is by the process of diffusion. **Diffusion** can be defined as a spontaneous process by which molecules move from a region of their greater concentration to a region of their lower concentration. Like all forms of work, the random movement of molecules that occurs in diffusion requires energy. This energy comes from kinetic activity of the solute and solvent molecules. This process is known as **passive diffusion**.

If a membrane is **permeable** to a substance it means the substance can easily pass through the membrane. If a substance X is at a different concentration on each side of a membrane permeable to X, then X will diffuse across the membrane from the side with a higher concentration of X to the side with a lower concentration of X until the concentration of X is the same on both sides of the membrane. If we could see the movement of X we would see as much X going in one direction as the other (**equilibrium**).

Diffusion through living cell membranes is typically restricted to water and other small molecules because large molecules cannot easily move through the passageways or “pores” present in the membrane. Because the movement of compounds across cell membranes is restricted to those below a certain size we call cell membranes **selectively permeable**.



OSMOSIS IS THE DIFFUSION OF WATER ACROSS A SELECTIVELY PERMEABLE MEMBRANE. Simply defined, osmosis is the movement of water through a selectively permeable membrane from a region of a higher concentration of water molecules to a region of a lower concentration of water molecules. In other words, water molecules move across the membrane from where the water is more concentrated (more pure or more water molecules) to where it is less concentrated (less pure or fewer water molecules). Note that osmosis refers to the movement of the solvent, water, not the solute. This phenomenon is illustrated in **Figure 3.1**.

With this information in mind, proceed with the following experiments.

In the diagrams to the left, assume that the membrane is selectively permeable and only allows water to pass through it. The solute, sodium chloride (NaCl), cannot pass through the membrane because its molecular size is too large. The terms hypotonic, isotonic, and hypertonic refer to the environments surrounding the circles. These terms are explained on page 3.7.

FIGURE 3.1 ✎
FOR EACH OF THE CONDITIONS ILLUSTRATED TO THE LEFT, DRAW AN ARROW IN THE DIRECTION WATER WOULD MOVE.

BROWNIAN MOTION DEMONSTRATION

Evidence of the molecular movement due to kinetic activity in the environment is provided by the phenomenon of **Brownian motion**. Molecules themselves are too small to be observed directly. However, larger visible particles will show the effects of molecular movement. One very easy way to see the effects of Brownian motion is by placing a drop of homogenized milk on a slide with a cover slip and viewing it with a compound microscope.

Examine the setup at the front of the lab. You should be able to see fat droplets jiggling in a random fashion. This jiggling motion of the droplets is called Brownian motion. Each fat droplet is composed of many molecules that do not dissolve in the surrounding watery fluid (this fluid contains the proteins and carbohydrates found in milk). These fat droplets (each consisting of many fat molecules grouped together) are being hit from all directions by the constant random motion of the molecules of the watery medium in which they are suspended. This results in the appearance of each fat droplet jiggling independently of the other fat droplets.

QUESTIONS ✎

1. Movement requires energy. What is the source of energy for the kinetic activity that results in Brownian Motion?

2. What would you predict about the motion if the room were cooler? _____

3. What would you predict about the motion if the room were warmer? _____

DIFFUSION EXPERIMENT 1: EFFECT OF MOLECULAR WEIGHT & TEMPERATURE ON THE RATE OF DIFFUSION

Obtain a Petri plate containing salt-free agar and proceed with the following as demonstrated by your instructor. (See **Figure 3.2**) Place the agar dish over the diagram and punch three holes in the agar by using the cork borer provided. Try not to crack the agar. Remove each agar plug from the borer before making another hole.

Being careful not to spill any solution on the surface of the agar, fill each hole with one of the solutions indicated in **Figure 3.2**. Fill each hole equally and just short of the top. Do not overfill the wells. Leave this plate at your desk undisturbed. Your instructor will also prepare a plate but s/he will place this one in the refrigerator. After 1.5 hours, use a ruler to measure the diameters of the dye circles (diffusion rings) and record your results in **Table 3.1**.

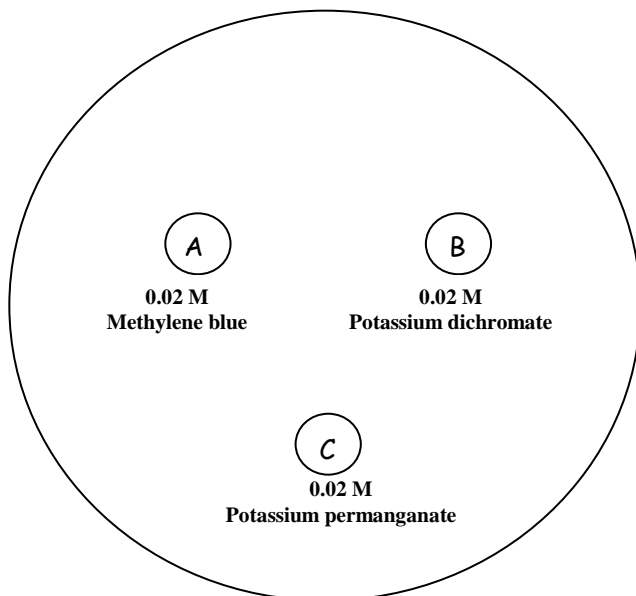


TABLE 3.1 ✎

EFFECTS OF MOLECULAR WEIGHT AND TEMPERATURE ON THE RATE OF DIFFUSION
DIAMETER (MM)

SOLUTION	MW	DIAMETER (MM)	
		REFRIGERATOR	ROOM TEMP
METHYLENE BLUE	319		
POTASSIUM DICHROMATE	294		
POTASSIUM PERMANGANATE	158		

FIGURE 3.2 Effect of molecular weight and temperature on the rate of diffusion.

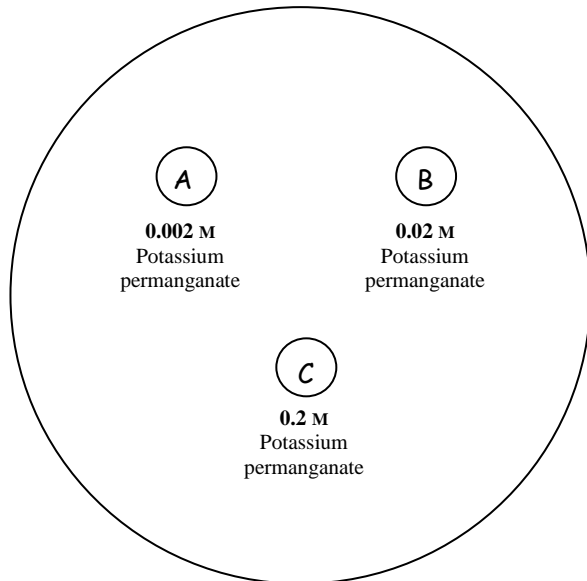
- A = Methylene blue (MW 319)
- B = Potassium dichromate (MW 294)
- C = Potassium permanganate (MW 158)

REFRIGERATOR TEMP = _____ °C

ROOM TEMPERATURE = _____ °C

Indicate the extent of diffusion with colored pencils after 90 minutes.

START TIME: _____



DIFFUSION EXPERIMENT 2: EFFECT OF SOLUTION CONCENTRATION ON RATE OF DIFFUSION

Obtain a Petri plate containing salt-free agar. Place the agar dish over the diagram (Figure 3.3) and punch three holes in the agar by using the cork borer provided. **Try not to crack the agar.** Remove each agar plug from the borer before making another hole.

Being careful not to spill any solution on the surface of the agar, fill each hole with the specific solution indicated in Figure 3.3. **Be sure you get the right solutions in the right holes. Double check before proceeding.** Fill the holes just short of the top, being careful not to spill any solution on the gel around the holes.

Cover the plate and place it on your desktop. Allow 90 minutes (1.5 hours) for the diffusion to occur, but **check the plate each 45 minutes** and record the diameter of the dye circles (diffusion rings) in Table 3.2.

FIGURE 3.3 Effect of concentration on the rate of diffusion of Potassium permanganate. **Indicate the extent of diffusion at 90 minutes. START TIME:** _____

TIME (MINUTES)	DIAMETER OF DIFFUSION RING		
	0.002 M	0.02 M	0.2 M
0			
45			
90			

QUESTIONS

- If a substance has high **molecular weight**, will its rate of diffusion that be lower than, higher than, or the same as that of substances with less weight? Write your *hypothesis* here:
 - Do the results of diffusion experiment 1 support or refute your hypothesis? Explain.
- If the temperature decreases, will the rate of diffusion increase, decrease, or stay the same? Write your *hypothesis* here:
 - Do the results of diffusion experiment 1 support or refute your hypothesis? Explain.
- If the starting concentration of a substance is higher, will its rate of diffusion be lower than, higher than, or the same as a substance of lower concentration? Write your *hypothesis* here:
 - Do the results of diffusion experiment 2 support or refute your hypothesis? Explain.

OSMOSIS – A SPECIFIC TYPE OF DIFFUSION

Osmosis is the net movement of water through a selectively permeable membrane from a region of higher concentration of water molecules to a region of lower concentration of water molecules. Energy for this movement, as in all cases of diffusion, is provided by kinetic activity present in the solute and solvent.

You will measure the rate of osmosis through an artificial, selectively permeable membrane called dialysis tubing. The dialysis tube is made of a cellulose-plastic compound that has been chemically engineered with tiny holes of a given size. These holes act as “pores” which allow water molecules to pass through more quickly than larger molecules such as sucrose (table sugar).

You will be making four “cells” out of the dialysis tubing, each containing a sucrose solution of a known concentration (20% w/v). Each cell will then be suspended in one of four different, unlabeled sucrose solutions. Depending on the concentration of the unlabeled solution, there will be a net movement of water either into or out of each cell. (Remember that the sucrose molecules are large and pass more slowly through the dialysis tubing than does the water.) By measuring the change in weight of each cell over time (due to its loss or gain of water as a result of osmosis), you will be able to determine the concentration of each of the four sugar solutions.

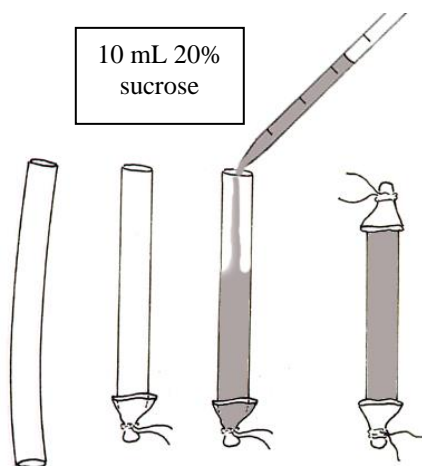
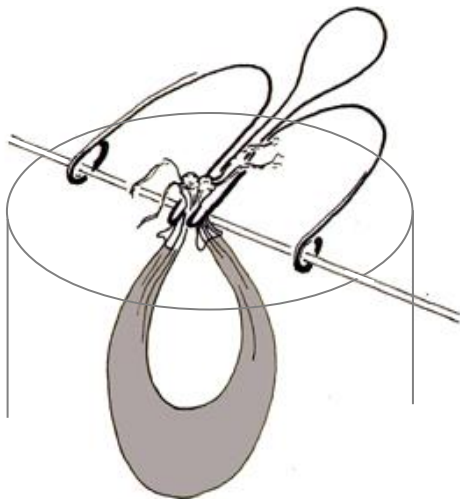


FIGURE 3.4 CREATING AN ARTIFICIAL “CELL”. *ABOVE* FILL AND TIE OFF FOUR LENGTHS OF DIALYSIS TUBES. *BELOW*: SUSPEND THE FILLED DIALYSIS TUBES INTO BEAKERS USING APPLICATOR STICKS AND W-CLAMPS.



PROCEDURE

- Each group should obtain 4 pre-soaked lengths of dialysis tubing from the front table. Additionally, on each table should be 4 250 mL beakers, 4 W-clamps, and 4 applicator sticks. **YOUR INSTRUCTOR WILL DEMONSTRATE HOW TO PREPARE AND FILL THE DIALYSIS TUBING.**
- After you have filled each bag with 10 mL of 20% sucrose solution and tied it off, clamp the two ends of the U-shaped bag in a W-clamp and, using an applicator stick, suspend the bag in any empty beaker. Mark each applicator stick with a different colored pencil so you can identify each bag.
- Label the beakers 1, 2, 3, and 4. You should have four labeled lab setups each in its own labeled beaker.
- Your instructor will demonstrate the use of the balance to make accurate weighings for your experimental setups (*i.e.*, the combined weight of each clamp-stick-bag unit).
- After blotting each bag with a tissue to remove any external moisture, weigh each of the four setups and record the weights in **Table 3.3** under the column “Initial Weight”. After all the setups are weighed, return to your desk and add the proper colored solution to each beaker (*i.e.*, solution 1 to beaker 1, *etc.*) so that the “cell” is entirely submerged in the liquid.
- After 15 minutes, remove all of the “cells” from their solutions, blot each bag with a tissue and then weigh each setup. Record each weight under the “15 min.” column in **Table 3.3**. After weighing all 4 setups, place them back into their proper solutions. Repeat these weighings at 15 minute intervals to obtain weights at time 30 minutes, 45 minutes and 60 minutes after the initial starting time. Record your data in **Table 3.3**.
- After completing the last weighing, discard the solutions in the sink (**do not return them to the solution bottles**), rinse off the equipment in tap water and return it to your lab table.
- Find the weight change of the bags after each 15 minute period by **subtracting the INITIAL weight** of each bag from the 15, 30, 45 and 60 minute weights for that bag. Record these changes in **Table 3.4**. Plot your results in **Figure 3.5**.
- The colored solutions have a sucrose concentration of 0%, 10%, 20%, and 40% (not particularly in that order). The sucrose solution in your bags is 20%. Based on your data of weight change in the dialysis tubing, determine the sugar concentration of each of the unknown solutions in which the bags were suspended. Answer Question 1a on page 3.6 during your experiments. After your data are collected, write the identity of each solution in the last column in **Table 3.4**.

TABLE 3.3 ✎ **START TIME:** _____

WEIGHT (G) AT TIME T (MINUTES)

SOL'N= SOLUTION	INITIAL WEIGHT AT TIME = 0	W E I G H T TIME = 15 MIN	W E I G H T TIME = 30 MIN	W E I G H T TIME = 45 MIN	W E I G H T TIME = 60 MIN
BAG IN SOL'N 1					
BAG IN SOL'N 2					
BAG IN SOL'N 3					
BAG IN SOL'N 4					

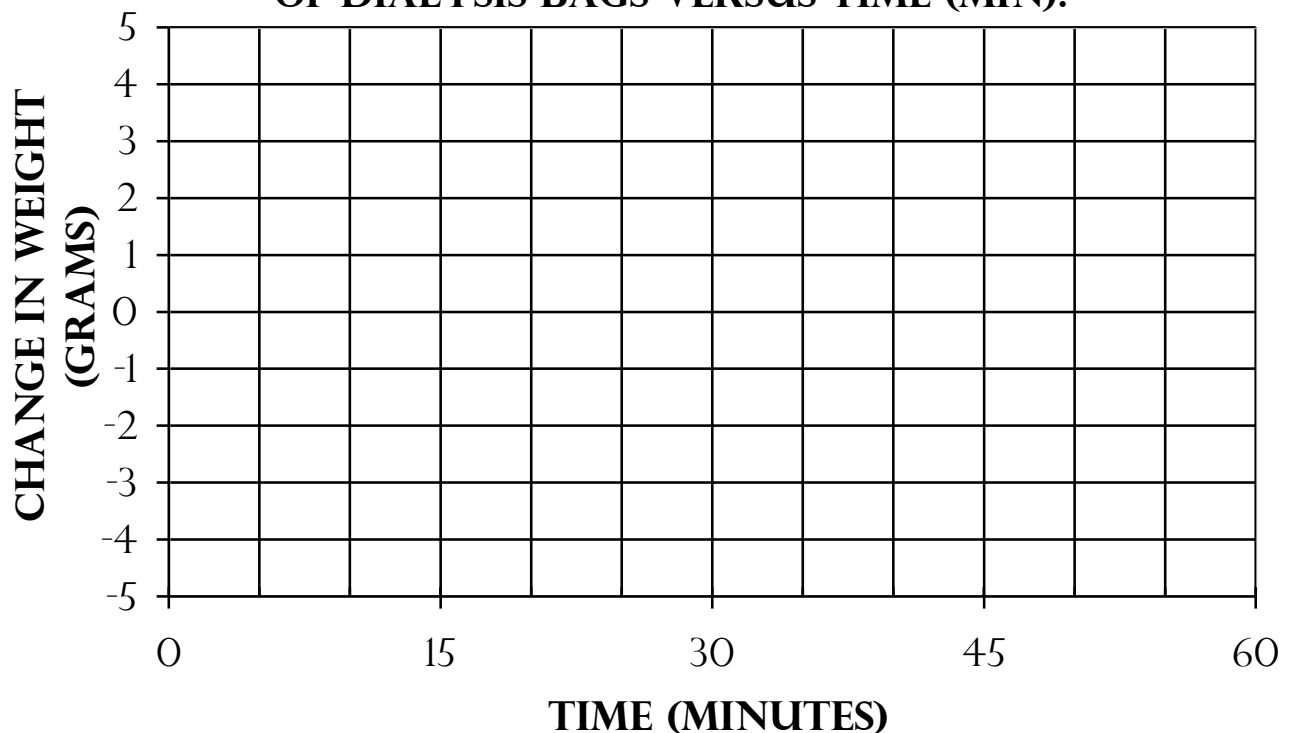
TABLE 3.4 ✎

CHANGE IN WEIGHT

(WEIGHT AT TIME T MINUS INITIAL WEIGHT)

	Δ WT (WT AT 15 MIN - INITIAL WT)	Δ WT (WT AT 30 MIN - INITIAL WT)	Δ WT (WT AT 45 MIN - INITIAL WT)	Δ WT (WT AT 60 MIN - INITIAL WT)	SOLUTION IDENTITY (%)
BAG IN SOL'N 1					
BAG IN SOL'N 2					
BAG IN SOL'N 3					
BAG IN SOL'N 4					

FIGURE 3.5 RATE OF OSMOSIS AS CHANGE IN WEIGHT (G) OF DIALYSIS BAGS VERSUS TIME (MIN).



Questions

1. a) While your experiment is running, in the space below, draw 4 beakers with cells (circles) inside of each; label each cell and each solution with the percent sucrose it contains. Then, use ARROWS to indicate your **hypothesis** for the NET direction of water movement: into the cell, out of the cell, or equal in both directions (equilibrium). Be sure your arrows do not enter or leave the beakers, since the water will not!

b) After the osmosis experiment is completed and you have filled in Figure 3.5, color in the solution for each of your four beakers above with the actual color that matches that percent sucrose, according to your analysis of your results. Below each beaker, write "data supports" or "data refutes" to indicate if your hypotheses were correct.

Using concepts from the osmosis experiments, explain the following:

2. Why does the lettuce become limp when you add salad dressing to the salad too long before the meal begins?
3. Why is salt used to preserve meat and other food?
4. If you were shipwrecked in the middle of the ocean, why would it be unwise to drink seawater? (Hint: seawater is approximately 3% and your body is approximately 1% salt concentration.)

The Effects of Osmosis on Cells

Solutes form weak bonds with water molecules thus inhibiting the movement of these water molecules across a semi-permeable membrane. Assume a cell is placed in a solution that has a lower concentration of dissolved solutes than exists in the cell. The external solution, therefore, has a higher concentration of free (unbound) water molecules than the interior of the cell. In this example, water will diffuse from a region of its higher concentration (outside the cell) to a place of its lower concentration (inside the cell) through the plasma membrane.

The osmotic process can be observed by placing plant or animal cells in aqueous solutions that are either: **hypotonic** (the concentration of solutes is less than that inside the cell), **isotonic** (the concentration of solutes equals that inside the cell), or **hypertonic** (the concentration of solutes is greater than inside the cell).

QUESTIONS

1. Provide a **hypothesis** for what would happen to cells if they are placed in a **hypotonic** environment.

2. What would happen to cells that are placed in a **hypertonic** environment?

Observe the instructor’s demonstration of the effect of salt solutions on onion cells. Sketch your observations below:

OBJECTIVE USED:

OBJECTIVE USED:

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ONION CELLS IN
HYPOTONIC SOLUTION

ONION CELLS IN
HYPERTONIC SOLUTION

QUESTION

Did your observations of onion cells agree with the predictions you made in Questions 1 and 2 above?

Clean-up:

- _____ **Do not dispose of remaining 20% sucrose solution in the flask. Return it to the tray on the side counter.**
- _____ **Pour colored sucrose solutions (those in the beakers labeled 1-4) into the the sink.**
- _____ **Wash and dry (no soap) all beakers and return to tray on the side counter.**
- _____ **Rinse and dry 10ml pipets and return to the tray on the side counter.**
- _____ **Do not dispose of wooden applicator sticks. Return to beaker on the tray on side counter.**
- _____ **Return all supplies to correct trays on the side counter.**
- _____ **Place used agar plates in bag marked “used plates” at the instructor’s table.**
- _____ **Put used dialysis tubing in the trash.**

LABORATORY NOTES
