

LABORATORY 1a: DIFFUSION & OSMOSIS (WATER POTENTIAL I)

OVERVIEW

In this laboratory you will investigate the processes of diffusion and osmosis in a model membrane system.

OBJECTIVES

Before you begin this lab you should understand:

- what the model membrane system is simulating
- the mechanisms of diffusion and osmosis and their importance to cells
- the effects of solute size and concentration gradients on diffusion across selectively permeable membranes
- the concept of molarity and its relationship to osmotic concentration

**also read the section on diffusion & osmosis in the text book **
(Chapter 8 pp. 145-146 in 6th ed. of Biology by Campbell, Reece & Mitchell)

At the completion of this lab you should be able to:

- relate osmotic potential to solute concentration and water potential

INTRODUCTION

You may not feel it but molecules inside of you are in constant motion. Many aspects of the life of a cell depend on the fact that atoms and molecules have kinetic energy and are constantly in motion. This kinetic energy causes molecules to bump into and rebound off each other and move in new directions. One result of this molecular motion is the process of diffusion.

Diffusion is the random movement of molecules from an area of higher concentration to an area of lower concentration. For example, if one were to open a bottle of hydrogen sulfide (H_2S has the odor of rotten eggs) in one corner of a room, it would not be too long before someone in the opposite corner would perceive the smell of rotten eggs. The bottle contains a higher concentration of H_2S molecules than the room does and, therefore, the H_2S gas diffuses from the area of higher concentration to the area of lower concentration. Eventually a dynamic equilibrium will be reached where the concentration of H_2S is approximately equal throughout the room and no NET movement of H_2S will occur from one area to the other.

Osmosis is a special case of diffusion. Osmosis is the passive diffusion of water across a membrane from a region of higher water potential to a region of lower water potential. Water potential is the measure of free energy of water in a solution.

Diffusion and osmosis do not entirely account for the movement of ions or molecules into and out of cells. One property of a living system is active transport. This process uses energy derived from ATP to move substances across the cell membrane. Active transport usually moves substances against a concentration gradient, from regions of low concentration into regions of higher concentration.

EXERCISE 1a-A: DIFFUSION

In this experiment you will measure diffusion of small molecules through dialysis tubing, a selectively permeable membrane. Small solute molecules (like glucose) and water molecules can move freely through a selectively permeable membrane, but larger molecules (such as sucrose) will pass through more slowly, or not at all. The movement of a solute through a selectively permeable membrane is called **dialysis**. The size of the pores in the dialysis tubing determines which substances can pass through.

A solution of glucose and starch will be placed inside a bag of dialysis tubing. The dialysis bag will then be placed inside a beaker of distilled water and IKI. After 30 minutes, the solution inside the dialysis tubing and the solution in the beaker will be tested for glucose and starch. The presence of glucose will be tested using Testape®, a strip of paper with green ends that change color in the presence of glucose. The presence of starch will be tested with Lugol's solution (IKI), a brown solution that turns blue in the presence of starch.

NOTES:

ON HOW TO HANDLE DIALYSIS TUBING:

- Do not place the dialysis tubing on the table. This will contaminate the tubing, possibly blocking pores.
- Do not let the dialysis tubing sit out in the air. This will dry out the tubing making it very fragile. The tubing should remain immersed in water until you are ready to use it.

ON HOW TO USE THE GLUCOSE REAGENT PAPER:

- Avoid contact with skin and mucous membranes; flush affected areas with copious amounts of water.
- Briefly (no longer than 1 second) dip test strip into the solution. Be sure that the patch on the test strip is totally immersed.
- Wait about a minute before reading your result. The colors are stable up to 3 minutes after immersion. Color changes that occur after 3 minutes from immersion are not of clinical value.
- A quantitative measurement is not necessary. On Table 1a.1, simply indicate the presence of glucose with either a (+) or (-).

MATERIALS

- ~ one 30-cm piece of 2.5-cm dialysis tubing
- ~ 15% glucose/1% starch solution
- ~ Testape® reagent paper (or other glucose testing paper)
- ~ one 250-mL beaker/plastic cup
- ~ Lugol's Solution (IKI [Iodine Potassium Iodide])
- ~ 10-mL graduated cylinder
- ~ funnel
- ~ string for tying dialysis bag

PROCEDURE

1. Fill a 250-mL beaker (or cup) two-thirds full with distilled water. Add approximately 4 mL of IKI to the distilled water and record the color of the solution in Table 1a.1. Test the solution with glucose reagent paper and record the results in Table 1a.1.
2. Obtain a 30-cm piece of 2.5-cm dialysis tubing that has been soaking in distilled water. Tie one end of the tubing with string to form a bag. Make sure that it doesn't leak. To open the other end of the bag, rub the end between your fingers until the edges separate.

3. Resuspend the 15% glucose/1% starch solution by swirling the flask until there is no visible solute. Using a funnel, pour 15mL of the solution in the dialysis bag. Tie off the other end of the bag, leaving a couple of inches for expansion of its contents. Record the color of the solution in Table 1a.1.
4. Immerse the bag in the solution in the beaker. Make sure the bag is **completely** covered by the solution.
5. Let the setup stand for approximately 30 minutes or until you see a distinct color change in the bag or in the beaker. While you are waiting:
 - (a) test the 15% glucose/1% starch solution with glucose reagent paper. There will be a beaker set up for this purpose. **Be sure all solute is completely suspended before testing.**
 - (b) start setting up for part B.
6. After 30 minutes, record the final color of the solution in the bag and the solution in the beaker in Table 1a.1.
7. Test the liquid in the beaker and in the bag with glucose reagent paper. To test the inside of the bag, cut off the top of the bag and dip the reagent paper into the solution. Record the results in Table 1a.1.

Table 1a.1

	INITIAL CONTENTS	SOLUTION COLOR		TESTAPE® RESULTS*	
		INITIAL	FINAL	INITIAL	FINAL
BAG					
BEAKER					

*for TESTAPE RESULTS - use (+) to indicate the presence of glucose and (-) to indicate the absence of glucose (do NOT give a quantitative measurement)

ANALYSIS OF RESULTS

1. Which substance(s) are entering the bag and which are leaving the bag? How do you know this? Support your answer with experimental evidence. Include concentration differences and bag pore size in your explanation.

2. **Quantitative** data uses **numbers** to measure observed changes. How could this experiment be modified so that quantitative data could be collected to show that water diffused into the dialysis bag?

3. Rank the following by relative size, beginning with the smallest: bag pores, glucose molecules, starch molecules, water molecules, IKI molecules.

EXERCISE 1a-B: OSMOSIS

In this experiment you will use dialysis tubing to investigate the relationship between solute concentration and the movement of water through a selectively permeable membrane by the process of osmosis. Because sucrose is significantly larger than glucose, it is unable to pass through this membrane.

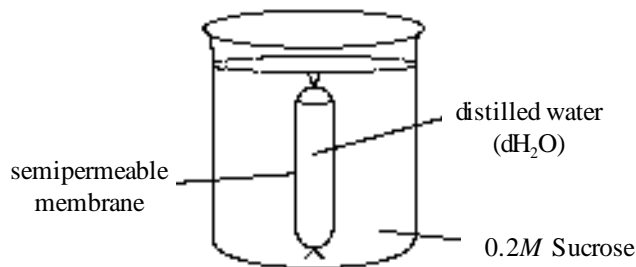
When two solutions have the same concentration of solutes, they are **isotonic** to each other (*iso* = same or equal; *tonic* refers to solute). If these solutions are separated by a selectively permeable membrane, water may move between them, but there will be **no net change** in the amount of water in either solution.

If two solutions differ in the concentration of solutes that each has, the one with more solute is **hypertonic** to the one with less solute (*hyper* = more, greater). The solution that has less solute is **hypotonic** to the one with more solute (*hypo* = under, less). **These are relative terms that are meaningful only in a comparative sense.**

Consider two solutions separated by a selectively permeable membrane. The solution that is hypertonic to the other must have a higher solute concentration, and therefore relatively less water. If the two solutions are at the same atmospheric pressure, the water potential of the hypertonic solution is less than the water potential of the hypotonic solution, so water will move from the hypotonic solution to the hypertonic solution.

Using Figure 1a.1: (1) indicate which solution is hypertonic and which is hypotonic;
(2) draw arrows to show the **net** movement of water

Figure 1a.1



MATERIALS

- ~ three 30-cm strips of presoaked dialysis tubing
- ~ string
- ~ the following solutions: (you will be assigned three by your teacher)
 - ~ distilled water
 - ~ 0.2-M sucrose
 - ~ 0.4-M sucrose
 - ~ 0.6-M sucrose
 - ~ 0.8-M sucrose
 - ~ 1.0-M sucrose
- ~ three beakers/plastic cups (250 mL)
- ~ paper towels

- ~ permanent marker
- ~ electronic balance

PROCEDURE

1. You will be assigned three solutions. For each solution, label a beaker/cup with the molarity. **Label using labeling tape. You are responsible for cleaning all glassware you use at the end of the lab.** Fill each beaker two-thirds full with distilled water.

NOTE: You will perform steps 2-6 for each solution you are assigned. Do one solution at a time. Do NOT let the dialysis tubing sit out in the air.

2. Obtain a piece of dialysis tubing. Tie off one end and pour 25 mL of one of your assigned solutions into the bag.
3. Remove the air from the bag by drawing the dialysis bag between two fingers. Tie off the other end of the bag, leaving about one-third as much room as the solution takes up.
4. Carefully blot the outside of each bag with a paper towel.
5. Weigh the bag and record the initial mass in Table 1a.2.
6. Immerse the bag in the appropriate beaker. **Be sure to completely submerge the bag.**
7. Let stand for approximately 30 minutes. [Note: You want to let each bag sit in solution for approximately the same time. If you put the second tubing in solution about 5 minutes after you put the first one in, take it out 5 minutes after you take the first one out. Why is this important?]
8. Remove the bags from the beakers and carefully blot each one. Determine the mass of each bag and record the final mass in Table 1a.2.

Table 1a.2: Individual Data

Contents of Dialysis Bag	Initial Mass	Final Mass	Difference	Percent Change in Mass
dH ₂ O				
0.2-M sucrose				
0.4-M sucrose				
0.6-M sucrose				
0.8-M sucrose				
1.0-M sucrose				

$$\% \text{ Change in Mass} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

SHOW ONE SAMPLE CALCULATION BELOW: (be sure to include units!)

for example:

0.6-M sucrose: initial mass = x grams
 final mass = y grams
 difference = (y - x) grams
 % change in mass = [(y - x) / x] grams = z grams

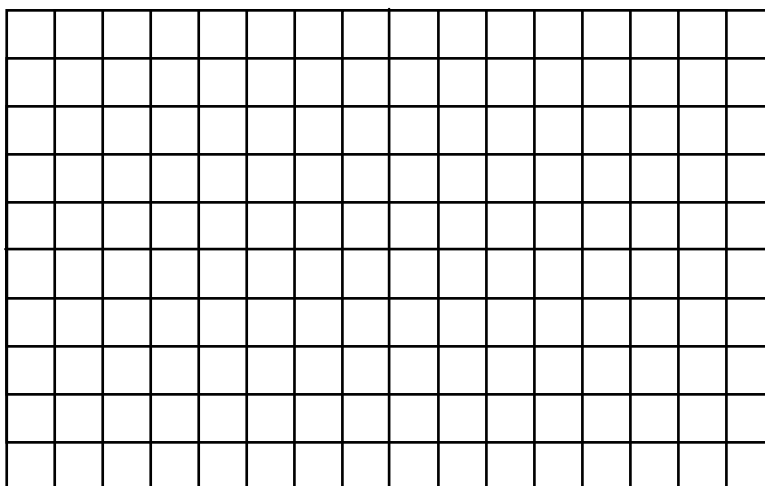
Table 1a.3: Class Data

group#	% Change in Dialysis Bag										
	1	2	3	4	5	6	7	8	9	10	ave.
dH ₂ O											
0.2-M											
0.4-M											
0.6-M											
0.8-M											
1.0-M											

ANALYSIS OF RESULTS

Graph the results for both your individual data and class data. Put the **independent variable** on the x-axis. Put the **dependent variable** on the y-axis. Be sure to label the x- and y- axis as well as indicate which line represents individual/class data.

TITLE _____



1. If you have a 1.0 molar solution of glucose, how could you prepare one liter of 0.2 molar solution of glucose? How could you prepare one liter of 0.02 molar solution? [Be specific. The only other item you have on hand is 5 liters of distilled water.]

2. What is the relationship between the percent change in mass and the molarity of sucrose within the dialysis bags?

3. Predict what would happen in this experiment if each of the bags (dH₂O, 0.2-M, 0.4-M, 0.6-M, 0.8-M, 1.0-M) were placed in a 0.4-M sucrose solution instead of dH₂O. [Remember: the dialysis tubing is impermeable to sucrose.] Explain your response. Use the terms hypertonic, hypotonic & isotonic in your explanation.

4. Why did you calculate the percent change in mass rather than simply using the change in mass?

5. If the initial mass of a bag is 20 g and the final mass is 18 g, calculate the percent change in mass. **Show your calculations.** Be sure to include units!

6. What type of solution would the bag above (#5) have been immersed in (iso-, hyper-, or hypotonic)? Rationalize your answer.
