

## Diffusion and Osmosis

Modified 2003 from *AP Bio Lab Manual*

### Introduction:

In this exercise you will measure diffusion of small molecules through dialysis tubing, an example of a semi-permeable membrane. The movement of a solute through a semi permeable membrane is called **dialysis (as well as diffusion)**. The size of the minute pores in the dialysis tubing determines which substance can pass through the membrane. A solution of glucose and starch will be placed inside a bag of dialysis tubing. Distilled water will be placed in a beaker, outside the dialysis bag. After 30 minutes have passed, 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 with **glucose test strips**. The presence of starch will be tested with **Lugol's** solution (iodine-potassium-iodide).

### Procedure:

1. Obtain a 30 cm piece of 2.5-cm dialysis tubing that has been soaking in water. Tie off one end of the tubing to form a bag. To open the other end of the bag, rub the end between your fingers until the edges separate.
2. Place 15 mL of the 15% glucose/1% starch solution in the bag. Tie off the other end of the bag, leaving sufficient space for the expansion of the bag's contents. Record the color of the solution in **Table 1.1**.
3. Test the 15% glucose / 1% starch solution in the bag for the presence of glucose. Your teacher may have you do a Benedict's test or use glucose Testape®. Record the results in **Table 1.1**.
4. Fill a 250 mL beaker or cup 2/3 full with distilled water. Add approximately 4 mL of Lugol's solution to the distilled water and record the color in **Table 1.1**. Test the solution for glucose and record the results in **Table 1.1**.
5. Immerse the dialysis bag in the beaker of water plus Lugol's.
6. Allow your set up to stand for approximately 30 minutes or until you see a distinct color change in the bag or the beaker. Record the final color of the solution in the bag and of the solution in the beaker in **Table 1.1**.
7. Test the liquid in the beaker and in the bag for the presence of glucose. Record the results in **Table 1.1**.

**Table 1.1** : Dialysis Tubing Changes

	Initial Contents	Initial Solution Color	Final Solution Color	Initial Presence of Glucose	Final Presence of Glucose
<b>Bag</b>	<b>15% Glucose &amp; 1% starch</b>				
<b>Beaker</b>	<b>H<sub>2</sub>O + IKI</b>				

### Analysis of Results:

1. Which substance(s) are entering the bag and which are leaving the bag? What experimental evidence supports your answer?
2. Explain the results you obtained. Include the concentration differences and membrane pore size in your discussion.
3. 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?
4. Based on your observations, rank the following by relative size, beginning with the smallest: glucose molecules, water molecules, IKI molecules, membrane pores, starch molecules.
5. What results would you expect if the experiment started with glucose and IKI solution inside the bag and only starch and water outside? Why?

### Osmosis:

In this experiment you will use dialysis tubing to investigate the relationship between solute concentration and the movement of water through a semi-permeable membrane by the process of osmosis. When two solutions have the same concentration of solutes, they are said to be **isotonic** to each other. If the two solutions are separated by a semi permeable membrane, water will move between the two solutions, 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 the less solute. The solution that has less solute is **hypotonic** to the one with more solute. These terms can only be used to compare solutions.

**Procedure:**

1. Obtain a 30 cm strip of pre-soaked dialysis tubing
2. Record mass
3. Pour approximately 25 mL of the following solutions into each bag.
 

Distilled water	0.6 M sucrose
0.2 M sucrose	0.8 M sucrose
0.4 M sucrose	1.0 M sucrose
4. Remove most of the air from the bags by drawing the dialysis bag between two fingers. Tie off the other end of the bag, leaving **sufficient space for the expansion**.
5. Rinse each bag gently with distilled water to remove any sucrose spilled during filling.
6. Carefully blot the outside of each bag and record the initial mass of each bag in **Table 1.2**.
7. Fill six 250 mL beakers 2/3 full with distilled water.
8. Immerse each bag in one of the beakers of distilled water and label the beaker to indicate the molarity of the solution in the dialysis bag. **Be sure to completely submerge each bag**.
9. Let stand for 30 minutes, remove the bags from the water, blot and determine the mass of
10. Record your group's results in **Table 1.2**. Obtain data from the other lab groups
11. Graph the results for both your individual data and class average on the following graph.

**Table 1.2 Dialysis Tubing Results**

Solution	Initial Mass	Final Mass	Mass Difference	% Change in Mass
<b>Distilled Water</b>				
<b>0.2 M</b>				
<b>0.4 M</b>				
<b>0.6 M</b>				
<b>0.8 M</b>				
<b>1.0 M</b>				

**To Calculate % Change in Mass:**

$$\frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

**Analysis of Results**

1. Explain the relationship between the change in mass and the molarity of sucrose within the dialysis bag.
2. Predict what would happen to the mass of each bag in this experiment if all the bags were placed in a 0.4 M sucrose solution instead of distilled water. Explain your response.
3. Why did you calculate the percent change in mass rather than using the change in mass?
4. A dialysis bag is filled with distilled water and then placed in a sucrose solution. The bag's initial mass is 20 g, and its final mass is 18 g. Calculate the percent change of mass, showing your calculations in the space below.
5. The sucrose solution in the beaker would have been \_\_\_\_\_ to the distilled water in the bag.

## Determining the Water Potential of Potato Cells

Water potential is defined as the tendency of water to diffuse from one region to another, Water potential is also a numerical value that varies according to temperature. In this activity you will determine the water potential of potato cells at room temperature and ambient pressure by placing cores of potato tissue in sucrose solutions of different concentrations and measuring the net movement of water in each case.

### Procedure

- Use cork borer to cut 5 potato cylinders. Cut each cylinder to a length of 3 cm
- Determine mass of all 5 potato cylinders together.
- Place cylinders in assigned solution
 

Distilled water	0.6 M sucrose
0.2 M sucrose	0.8 M sucrose
0.4 M sucrose	1.0 M sucrose
- Let stand overnight
- Record temperature, remove cylinders, and determine mass of all 5 cylinders
- Determine percent change in mass
- Graph class results
- Determine the molarity of the sucrose solution in which the mass of the potato cores does not change (Determine this value from the graph...draw a line of best fit: The point at which this line crosses the x-axis represents the molar concentration of sucrose with a water potential that is equal to the potato tissue water potential. At this concentration there is no net gain or loss of water from the tissue
- Calculate the osmotic potential of this sucrose solution using the following formula

$$\pi_p = -iCRT$$

Where  $i$  = ionization constant (this is 1 for sucrose because sucrose does not ionize in water)

$C$  = osmotic molar concentration (determined from graph above)

Pressure constant ( $R = 0.0831$  liter /bas/mole  $^{\circ}K$ )

$T$  = temperature  $^{\circ}K$  (remember  $273 + ^{\circ}C$  solution)

Units of osmotic potential will be bars (This is a measure of pressure)

- The water potential of the potato cells is equal to the osmotic potential of the sucrose solution at equilibrium.

Table 1-3: Potato Osmosis Results

Solution	Temperature $^{\circ}C$	Initial Mass	Final Mass	Percent Change in Mass*
Distilled water	24	5.06		
0.2 M sucrose	24	5.08		
0.4 M sucrose	24	5.06		
0.6 M sucrose	24	5.03		
0.8 M sucrose	24	5.07		
1.0 M sucrose	24	5.03		

### To Calculate % Change in Mass:

$$\frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

### Analysis of Results

- If the potato is allowed to dehydrate by sitting in the open air, would the water potential of the potato cells become higher or lower? (Explain)
- If a plant cell has a lower water potential than its surrounding environment and if pressure is equal to zero, is the cell hypertonic (in terms of solute concentration) or hypotonic to its environment?

**Table 1.2 Dialysis Tubing Results**

<b>Solution</b>	<b>Initial Mass</b>	<b>Final Mass</b>	<b>Mass Difference</b>	<b>% Change in Mass</b>
<b>Distilled Water</b>	4.63	4.87		
<b>0.2 M</b>	3.61	3.66		
<b>0.4 M</b>	5.48	5.03		
<b>0.6 M</b>	6.42	5.68		
<b>0.8 M</b>	5.07	4.49		
<b>1.0 M</b>	<b>6.09</b>	<b>4.86</b>		
<b>Distilled Water</b>	<b>4.61</b>	<b>4.93</b>		
<b>0.2 M</b>	<b>6.12</b>	<b>6.36</b>		
<b>0.4 M</b>	<b>4.89</b>	<b>4.58</b>		
<b>0.6 M</b>	<b>4.97</b>	<b>4.64</b>		
<b>0.8 M</b>	<b>4.94</b>	<b>4.66</b>		
<b>1.0 M</b>	<b>5.77</b>	<b>4.90</b>		

**To Calculate % Change in Mass:**

$$\frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

**Table 1-3: Potato Osmosis Results**

<b>Solution</b>	<b>Temperature °C</b>	<b>Initial Mass</b>	<b>Final Mass</b>	<b>Percent Change in Mass*</b>
Distilled water	24	5.06	5.99	
0.2 M sucrose	24	5.08	5.31	
0.4 M sucrose	24	5.06	3.80	
0.6 M sucrose	24	5.03	2.95	
0.8 M sucrose	24	5.07	2.95	
1.0 M sucrose	24	5.03	3.82	

**To Calculate % Change in Mass:**

$$\frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$