Big Idea 2

Cellular Processes: Energy and Communication

Baker's Dozen Lab 4: Diffusion and Osmosis

Objectives

Before doing this lab you should understand the effects of solute size and concentration gradient on diffusion across selectively permeable membranes and the concept of molarity and its relationship to osmotic concentration.

After doing this lab you should be able to measure the water potential of a solution in a controlled experiment, determine the osmotic concentration of living tissue from experimental data, describe the effects of water gain or loss in animal and plant cells, and relate osmotic potential to solute concentration and water potential.

Introduction

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes are **selectively permeable**, meaning they allow some substances to move across while preventing others from doing so.

The cellular environment is aqueous, meaning that the solvent in which the solutes, such as salts and organic molecules, dissolve is water. Water may pass slowly through the membrane by osmosis or through specialized protein channels called **aquaporins**. Aquaporins allow the water to move more quickly than it would through osmosis. Most other substances, such as ions, move through protein channels, while larger molecules, such as carbohydrates, move through transport proteins.

The simplest form of movement is **diffusion**, in which solutes move from an area of high concentration to an area of low concentration; diffusion is directly related to molecular kinetic energy. Diffusion does not require energy input by cells. The movement of a solute from an area of low concentration to an area of high concentration requires energy input in the form of ATP and protein carriers called pumps.

Water moves through membranes by a specialized type of diffusion called **osmosis**. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential to areas of low potential. Solutes decrease the concentration of free water, since water molecules surround the solute molecules. The terms **hypertonic, hypotonic,** and **isotonic** are used to describe solutions separated by selectively permeable membranes.

In nonwalled cells, such as animal cells, the movement of water into and out of a cell is affected by the relative solute concentration on either side of the plasma membrane. As water moves out of the cell, the cell shrinks. As water moves into the cell, it swells and may eventually burst. In walled cells, including fungal and plant cells, osmosis is affected not only by the solute concentration, but also by the resistance to water movement in the cell by the cell wall. This resistance is called **turgor pressure**. The presence of a cell wall prevents the cell from bursting as water enters; however pressure builds up inside the cell and affects the rate of osmosis.

Part A: Demonstration

In this demonstration, you will observe diffusion of small molecules through dialysis tubing, an example of a selectively permeable membrane. Small solutes and water can move freely through it, but larger molecules cannot. The size of the minute pores in the dialysis tubing determines which substances can pass through it. A solution of glucose and starch will be placed inside the dialysis bag, while distilled water and iodine will be placed in the beaker surrounding the bag.

Table 4.1:

	Initial Contents	Solution Color		Presence of Glucose	
		Initial	Final	Initial	Final
Bag	15% glucose & 1% starch				
Beaker	H_2O & IKI				

Analysis of Results:

1. To what is the dialysis bag selectively permeable? Write a CER using the previous data table and vocabulary terms.

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2. Based on your observations, rank the following by relative size, beginning with the **SMALLEST**: glucose molecules, starch molecules, water molecules, iodine molecules, membrane pores.

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3. What results would you expect if the experiment started with the glucose and iodine solution inside the bag and the starch and water outside? Why? ____

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Part B: Modeling Osmosis

In this experiment, you will identify unknown solutions based on their percent change in mass due to osmosis.

Materials:

- Unknown solution A
- Unknown solution B
- 0.5 M sucrose solution
- Balances
- Scissors
- 20-cm long dialysis tubes
- Thread
- Cups

Procedure

1. Working in pairs, prepare one bag ½ full with each of the unknown solutions.
2. Explain the evidence you are looking for to identify which solution is which
3. Make dialysis bags as demonstrated by your teacher. Soak the bags in water to help open them up. Tie one end shut with thread, fill it up with the appropriate solution, and tie the other end shut with thread. **Be sure to leave some room in the bag for water to move in or out.**
4. Mass the bags using a balance, and record it in the data table 4.2.
5. Label the cups with the solution pair. Fill each cup (about 2/3 full) with the appropriate solution and place the appropriate dialysis bag into each cup.
6. After 30 minutes, remove the bag from the cup. Pat it dry with a paper towel, and re-mass it. Record this information in the data table 4.2.
7. Calculate the percent change in mass using the formula: (final mass-initial mass)/initial mass x 100. Record this information in the data table 4.2.

Explanation of Evidence Needed: ____

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Data Table 4.2:

Solution Pair	Initial Mass (grams)	Final Mass (grams)	Percent Change	Class Ave % Change
Sucrose/Solution A				
Sucrose/Solution B				

Analysis of Results:

1. Which solution gained mass? Which solution lost mass?
____
____
2. Write a CER about the solutions in each bag. ____
____
____
____
3. You are in the hospital and need IV fluids. Reading the label on the IV bag you notice it has salts in it. Why is that?
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____
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Part C: Determining Molarity of Living Cells

In this experiment, you will use solutions of known molarity to determine the solute concentration (molarity) of a living cell. Which do you think has a higher solute concentration – a potato or an apple?

Materials:

solutions of 0 M, 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1 M sucrose
different kinds of potatoes
plastic wrap
scalpels
cups
balance

Procedure:

1. Using the scalpel, cut 24 rectangular pieces of the food sample you are testing. **Be sure to trim off the skin**.
2. Fill each of the six cups (about 2/3 full) with one of the six different solutions.
3. Mass four rectangular pieces and record the initial mass in data table 4.3 next to the correct solution. Place those pieces into that solution's cup.
4. Repeat step 3 for each of the cups until all 24 rectangular pieces are placed in cups.
5. Cover each cup with plastic wrap. Place them somewhere safe around the room and leave overnight.
6. The next day, mass each set of rectangular pieces and record it in data table 4.3.
7. Calculate the percent change in weight according to the same formula as part B.
8. Graph the class averages for **BOTH** food samples on graph 4.1.

Table 4.3: (Record your table and class data here, but be sure to graph all four classes data)

Solution in Cup	**Initial Mass (grams)**	**Final Mass (grams)**	**Percent Change**	**___________ % Change Class Average**	**____________ % Change Class Average**
0 M					
0.2 M					
0.4 M					
0.6 M					
0.8 M					
1.0 M					

Determining Molarity of Food Sample

The molarity of the apple or potato can be determined as it will equal the molarity of the sucrose solution where the mass of the food sample does not change. To find this, use the graph of the percent changes in mass on the next page. **The point at which this line crosses the x-axis represents the molar concentration of sucrose that is equal to the molar concentration of the food sample.** At this concentration, there is no net gain or loss of water from the tissue. Write this concentration in the space below.

Molar concentration of __________ sample = ____________________ M

Molar concentration of __________ sample = ____________________ M

Analysis of Results:

1. Identify the independent variable of this experiment. ____

2. Identify the dependent variable of this experiment. ____

3. Write a CER about which food sample has the highest molarity. ____
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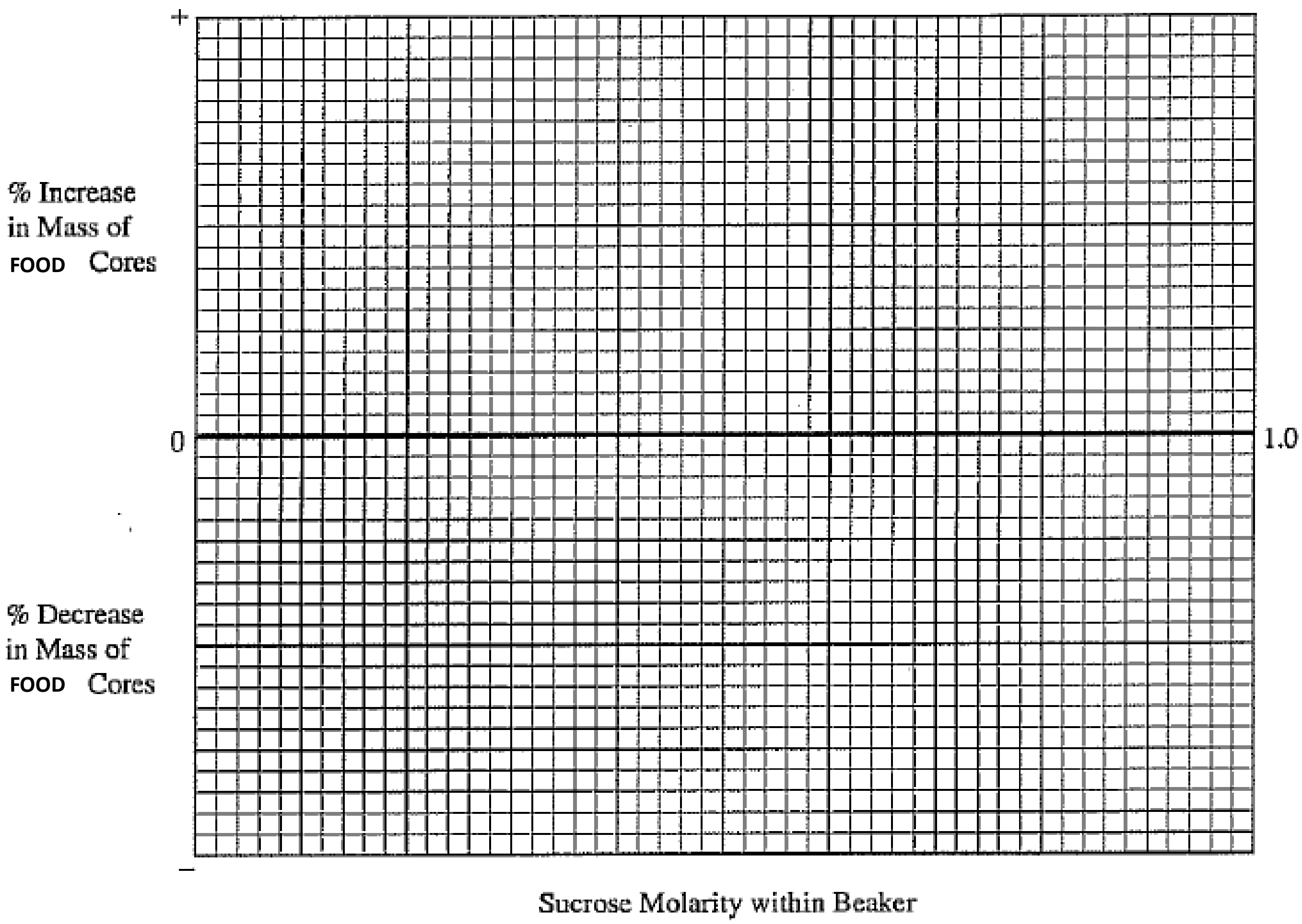
____
____
____

4. Why was the percent change in mass used instead of raw mass gained or lost to compare data between the two types of food? ____
____
____
____

Graph 4.1 **Title:** ____________________



Part D: Understanding Water Potential

Water potential predicts which way water diffuses through plant tissues and is abbreviated by the Greek letter psi (ψ). Water potential is the free energy per mole of water and is calculated from two major components: 1) the solute potential (ψ_s), which is dependent on solute concentration and 2) the pressure potential (ψ_p), which results from the exertion of pressure – either positive or negative (tension) – on a solution. The solute potential is also called the osmotic potential.

$$\psi = \psi_p + \psi_s$$

Water potential = pressure potential + solute potential

Water moves from an area of higher water potential or higher free energy to an area of lower water potential or lower free energy. **Water potential measures the tendency of water to diffuse** from one compartment to another compartment.

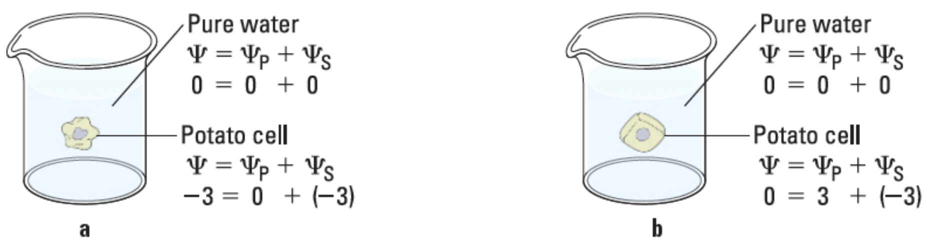
The water potential of pure water in an open beaker is zero (ψ=0) because both the solute and pressure potentials are zero ($\psi_s = 0$; $\psi_p = 0$). An increase in positive pressure raises the pressure potential and the water potential. The addition of solute to the water lowers the solute potential and therefore decreases the water potential. This means that a solution at atmospheric pressure has a negative water potential due to the solute.

The **solute potential (ψ_s) = -iCRT**, where i is the ionization constant, C is the molar concentration, R is the pressure constant (R=0.0831 liter-bars/mole-K), and T is the temperature in K (273 + ^{o}C).

To calculate the water potential of a 0.15 M solution of sucrose at atmospheric pressure (ψ_p=0) and 25°C, you first have to calculate the solute (osmotic) potential. The ionization constant (i) for sucrose is 1 since it doesn't ionize in water. The pressure constant is always 0.0831 liter-bars/mole-K, and the temperature is 298 K (273+25°C). Multiply all those together and the solute (osmotic) potential is -3.7 bars. Since the pressure potential is zero, the water potential is also -3.7 bars. A bar is a metric measure of pressure and is the same as 1 atmosphere at sea level. A 0.15 M NaCl solution contains 2 ions, Na^+ and Cl^-; therefore i = 2. If it is at the same temperature, the water potential would equal -7.4 bars.

When a cell's cytoplasm is separated from pure water by a selectively permeable membrane, water moves from the surrounding area, where the water potential is higher (ψ=0), into the cell, where water potential is lower because of solutes in the cytoplasm (ψ is negative). It is assumed that the solute is not diffusing (Figure 1a). The movement of water into the cell causes the cell to swell, and the cell membrane pushes against the cell wall to produce an increase in pressure. This pressure, which counteracts the diffusion of water into the cell, is called turgor pressure.

Over time, enough positive turgor pressure builds up to oppose the more negative solute potential of the cell. Eventually, the water potential of the cell equals the water potential of the pure water outside the cell (ψ of cell = ψ of pure water = 0). At this point, a dynamic equilibrium is reached and net water movement ceases (Figure 1b).



Figures 1a-b. Plant cell in pure water. The water potential was calculated at the beginning of the experiment (a) and after water movement reached dynamic equilibrium and the net water movement was zero (b).

If solute is added to the water surrounding the plant cell, the water potential of the solution surrounding the cell decreases. If enough solute is added, the water potential outside the cell is equal to the water potential inside the cell, and there will be no net movement of water. However, the solute concentrations inside and outside the cell are not equal, because the water potential inside the cell results from the combination of both the turgor pressure (ψ_p) and the solute pressure (ψ_s). (See Figure 2)

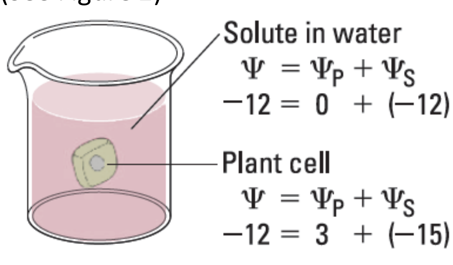


Figure 2. Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not equal.

If more solute is added to the water surrounding the cell, water will leave the cell, moving from an area of higher water potential to an area of lower water potential. The water loss causes the cell to lose turgor. A continued loss of water will cause the cell membrane to shrink away from the cell wall, and the cell will plasmolyze.

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Practice Problems

Calculate the solute potential of a 0.2 M NaCl solution at 25°C using the equation $\psi_s = -iCRT$. Show your work below.

If the concentration of NaCl inside a plant cell is 0.15 M, which way will the water diffuse if the cell is placed into a 0.2 M NaCl solution? ____

Use the molarity of the food sample determined from the graph and calculate the solute potential using the equation $\Psi = -iCRT$. Show your work below. (Room temperature is usually 25°C). Do this for BOTH food samples.

Analysis of Results

1. If a potato core is allowed to dehydrate by sitting in the open air, would the water potential of the potato cells decrease or increase? Why?

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2. 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? Will the cell gain water or lose water? Explain your response.

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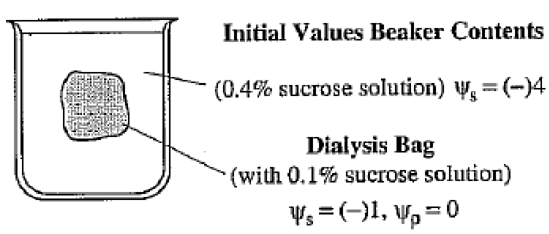

3. In the figure above, the beaker is open to the atmosphere. What is the pressure potential (ψ_p) of the system?

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4. In the figure above, where is the greatest water potential – beaker or dialysis bag? ________________________

5. To where will the water diffuse? Into or out of the bag? ____