DIFFUSION AND OSMOSIS – Part 1

What causes plants to wilt if they are not watered?

BACKGROUND

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes, including the plasma and organelle membranes, are selectively permeable. Membranes are phospholipid bilayers containing embedded proteins; the phospholipid fatty acids limit the movement of water because of their hydrophobic characteristics.

The cellular environment is aqueous, meaning that the solutes (e.g., salts, organic molecules) dissolve in water, which is the solvent. 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, including 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 diffusion; the movement of water through membranes is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high free water concentration) and low solute concentration to areas of low potential (low free water concentration) and high solute concentration. 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. A hypertonic solution has a higher solute concentration and a lower water potential as compared to the other solution; therefore, water will move into the hypertonic solute concentration and a higher water potential than the solution on the other solute of the membrane; water will move down its concentration gradient into the other solution. Isotonic solutions have equal water potentials.

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; if 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 cells from bursting as water enters; however, pressure builds up inside the cell and affects the rate of osmosis.

Water movement in plants is important in water transport from the roots into the shoots and leaves. You likely will explore this specialized movement called transpiration in another lab investigation.

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 ($\psi = 0$) 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 + °C).

A 0.15 M solution of sucrose at atmospheric pressure ($\psi_P = 0$) and 25°C has an osmotic potential of -3.7 bars and a water potential of -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 and the water potential = -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 $(\psi = 0)$, into the cell, where water potential is lower because of solutes in the cytoplasm $(\psi \text{ is negative})$. 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.

- Calculate the solute potential of a 0.1 M NaCl solution at 25°C. If the concentration of NaCl inside the plant cell is 0.15 M, which way will the water diffuse if the cell is placed into the 0.1 M NaCl solutions?
- 2) What must the turgor pressure equal if there is no net diffusion between the solution and the cell?

Learning Objectives

- 1) To investigate the relationship among surface area, volume, and the rate of diffusion
- 2) To design experiments to measure the rate of osmosis in a model system
- 3) To investigate osmosis in plant cells

- 4) To design an experiment to measure water potential in plant cells
- 5) To analyze the data collected in the experiments and make predictions about
- 6) molecular movement through cellular membranes
- 7) To work collaboratively to design experiments and analyze results
- 8) To connect the concepts of diffusion and osmosis to the cell structure and function

General Safety Precautions

You must wear safety glasses or goggles, aprons, and gloves because you will be working with acids and caustic chemicals. The HCl and NaOH solutions will cause chemical burns, and you should use these solutions in spill-proof trays or pans. Follow your teacher's instructions carefully. Do not work in the laboratory without your teacher's supervision. Talk to your teacher if you have any questions or concerns about the experiments.

THE INVESTIGATIONS

This investigation consists of three parts. In Procedure 1, you use artificial cells to study the relationship of surface area and volume. In Procedure 2, you create models of living cells to explore osmosis and diffusion. You finish by observing osmosis in living cells (Procedure 3). All three sections of the investigation provide opportunities for you to design and conduct your own experiments.

Getting Started

These questions are designed to help you understand kinetic energy, osmosis, and diffusion and to prepare for your investigations.

1) What is kinetic energy, and how does it differ from potential energy?

2) What environmental factors affect kinetic energy and diffusion?

3)	How do these factors alter diffusion rates?
4)	Why are gradients important in diffusion and osmosis?
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5)	What is the explanation for the fact that most cells are small and have cell membranes with many convolutions?
6)	Will water move into or out of a plant cell if the cell has a higher water potential than the surrounding environment?
	

7)	What would happen if you applied saltwater to a plant? Why?
8)	How does a plant cell control its internal (turgor) pressure?

■ Procedure 1: Surface Area and Cell Size

Cell size and shape are important factors in determining the rate of diffusion. Think about cells with specialized functions, such as the epithelial cells that line the small intestine or plant root hairs.

• What is the shape of these cells?

• What size are the cells?

• How do small intestinal epithelial and root hair cells function in nutrient procurement?

Materials

• 2% agar containing NaOH and the pH-	Squares of hard, thin plastic (from
indicator dye phenolphthalein	disposable plates); unserrated knives; or
 1% phenolphthalein solution 	scalpels from dissection kits

• 0.1M HCI	Metric rulers
• 0.1 M NaOH	 Petri dishes and test tubes
	 2% agar with phenolphthalein preparation

Step 1 - Understanding Your Materials

Place some phenolphthalein in two test tubes. Add 0.1 M HCl to one test tube, swirl to mix the solutions, and observe the color. Using the same procedure, add 0.1M NaOH to the other test tube. Remember to record your observations as you were instructed.

- 1) Which solution is an acid?
- 2) Which solution is a base?
- 3) What color is the phenolphthalein when mixed with the acid?
- 4) What color is the phenolphthalein when mixed with the base?

Alternative Method

Mix one packet of unflavored gelatin with 237 mL of water: add 2.5 mL 1% phenolphthalein and a few drops of 0.1 M NaOH. The solution should be bright pink. Pour the gelatin mixture into shallow pans and refrigerate overnight. You may use white vinegar in place of the 0.1 M HCI.

Step 2 Using a dull knife or a thin strip of hard plastic, cut three blocks of agar of different sizes.

These three blocks will be your models for cells.

- 1) What are the dimensions of each of your three cells? Record it in the data table provided.
- 2) What is the surface area of each of your three cells? Record it in the data table provided.
- 3) What is the total volume of each of your three cells? Record it in the data table provided.

Form a **hypothesis** that relates cell surface area, cell volume and rate of diffusion throughout the *entire* cell:

Using your hypothesis, make a **prediction**: If you put each of the blocks into a solution, into which block would that solution diffuse throughout the entire block fastest? Slowest? How do you explain the difference?

Designing and Conducting Your Investigation

Using the materials listed earlier, design an experiment to test your hypothesis you just made regarding the relationship of surface area and volume in the artificial cells to the diffusion rate using the phenolphthalein–NaOH agar and the HCl solution. Once you have finished planning your experiment, have your teacher check your design. When you have an approved design, run your experiment and record your results. Do your experimental results support your predictions?

Identify the following in your experiment:

Dependent variable (Y-axis)

Independent variable (X-axis)

Constants (give at least 4)

Some helpful tips to analyze your data:

- 1) Using a metric ruler, measure the distance that the white vinegar diffused into each cube. (Distance from surface).
- 2) Calculate the <u>rate of diffusion</u> for each cube in centimeters per minute (cm/min).
- 3) Calculate the volume of the portion of each cube which has not changed color (in other words, the portion of the cube that is still pink).
- 4) Calculate the percentage of the cube that has changed color. This will provide the *extent of diffusion* into each cube as a percent of the total volume:

	Total Cube Volume -	Volume of cube that has not changed color	
Extent of Diffusion =			X 100
Extent of Diffusion =			, <u> </u>

Total Cube Volume



Using the above diagram and dimensions, the equation becomes:

Extent of Diffusion =
$$\frac{S^3 - (S - 2X)^3}{S^3} \times 100$$

- 5) Make six graphs. Make sure each graph has an appropriate title, labeled with a Figure number, both axes have appropriate intervals, both axes are labeled, both axes have units of measurement:
 - a. Rate of diffusion relative to cell volume
 - b. Rate of diffusion relative to cell surface area.
 - c. Rate of diffusion relative to surface area/volume ratio.
 - d. Extent of diffusion relative to cell volume.

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- e. Extent of diffusion relative to cell surface area.
- f. Extent of diffusion relative to surface area/volume ratio

Cube dimensions (cm X cm X cm)	Surface Area (cm ²)	Volume (cm ³)	SA/Vol ratio	Distance vinegar diffused (cm)	Time allowed for diffusion (min)	Rate of diffusion (cm/min)	Volume of Cube NOT changed color (still pink) (cm ³)	Extent of Diffusion (% of cube changed color)

Table 1 – Cube Dimensions and Diffusion Rate

Let S =length of a side of the cube

Let X = distance vinegar penetrated (clear)

Surface area is calculated by $6S^2$ (where S = length of side of cube)

Volume is calculated by S^3

SA/Vol ratio is calculated by dividing surface area by volume.

Distance vinegar diffused is obtained by measuring with a ruler from a surface of the cube towards the center where the color of the cube is now clear. Let this value = X.

Rate of diffusion is calculated by dividing distance vinegar diffused by the time allowed for diffusion.

Volume of cube NOT changed color (still pink) is calculated as follows = $(S - 2X)^3$

Extent of Diffusion (% of cube changed color and is now clear) is calculated as follows = $[S^3 - (S - 2X)^3] / S^3 * 100$

Analysis Questions

1) Analyze *each* of your six graphs. What relationship is shown by *each* graph?

2) Restate your hypothesis. Does the data support your hypothesis? Explain by citing supporting data as evidence.

3) What size are generally most cells? Why?

4) What should the surface area/volume ratio be in order for diffusion throughout the entire cube be the fastest? Slowest?

- 5) Give an example of how the rate of diffusion is important in the following body systems and describe the shapes and sizes of cells and tissues involved:
 - a. Respiratory system

b. Circulatory system

c. Nervous system

d. Digestive system

e. Excretory system

- 6) Describe the function of each structure. Describe the shapes and sizes of cells and tissues that make up the structures. Why is the rate of diffusion important in these structures? Describe how the shapes and sizes of cells and tissues relate to rate of diffusion.
 - a. Plant root hairs

b. Plant leaves

c. Fungus hyphae and mycelia

d. Earthworm skin

e. Fish gills