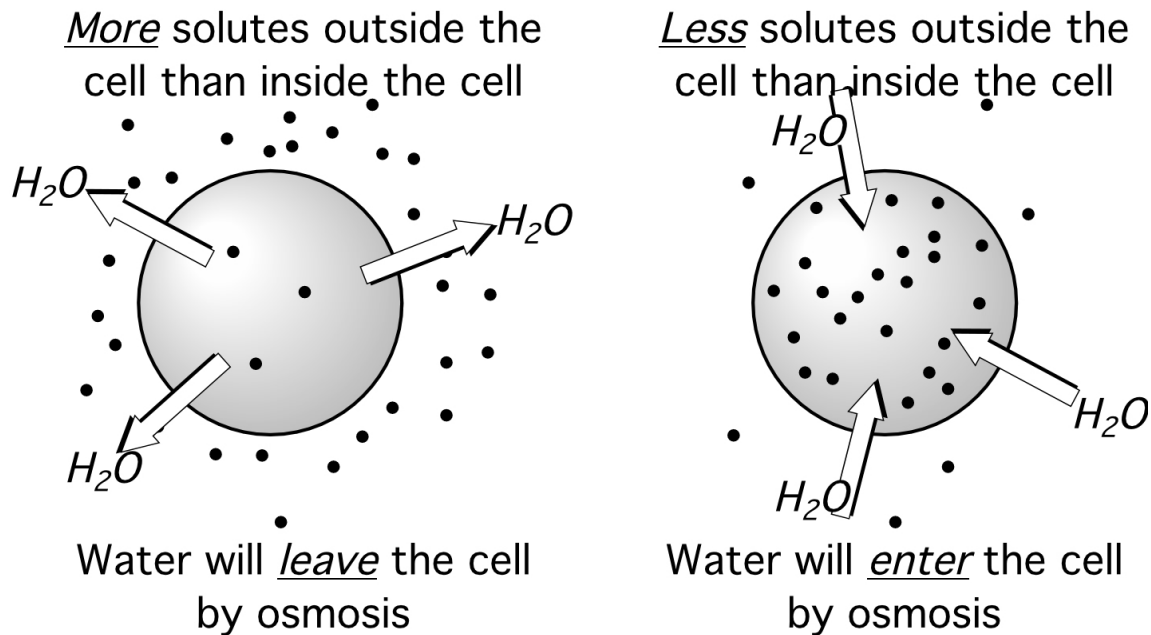


Diffusion and Osmosis

a) Introduction to osmosis

Osmosis is the movement of water through a membrane. To be more exact, osmosis is defined as the movement of water across a semi-permeable membrane towards whichever side of the membrane has the highest solute concentration. (A semi-permeable membrane is one that allows water molecules to pass through but not the solute molecules).



The membranes of most cells are semi-permeable. This means that a cell will gain water if it has a higher solute concentration than the liquid that surrounds it, and a cell will lose water if it has a lower solute concentration than the liquid that surrounds it.

A liquid that has a higher solute concentration than a cell (and therefore will cause the cell to lose water by osmosis) is called a hypertonic solution. A liquid that has a lower solute concentration than a cell (and therefore will cause the cell to gain water by osmosis) is called a hypotonic solution. A liquid that has the same solute concentration as a cell (and therefore will not cause the cell to gain or lose water) is called an isotonic solution.

In the next activity, you will investigate the effect of solute concentration on osmosis rate by constructing “cells” using dialysis membranes. Although you can’t see it with the naked eye, the dialysis membrane has millions of tiny holes. Water molecules are small enough to easily pass through, but larger solute molecules can’t pass. Thus, the membrane functions as a semi-permeable membrane (similar to a real cell’s membrane).

b) The effect of solute concentration on osmosis rate

Do this activity only if you are in one of the Right-side groups of the lab. If you are a Left-side lab group, do activity (c) instead. But both groups should give their data to the instructor when the activity is finished.

All cells have solutes inside. Suppose a cell was put into pure water (which has a lower solute concentration than the cell). The principle of osmosis states that water will move into the cell, but is the rate of water movement affected by the cell's solute concentration? In other words, if we could increase the solute concentration inside the cell, would the cell gain more water? To test this question, you will make two "cells" out of dialysis tubing and fill them with solutes. The solutes will two different concentrations of sucrose (a small disaccharide molecule) or albumin (a large protein molecule). The concentration of these solutes is given in % (which means grams of the solute per 100 ml of solution).

1) On the front desk is a supply of dialysis tubes that are soaking in a beaker of water. Obtain one piece of dialysis tubing from the beaker. Also obtain four plastic clamps and two empty 300 ml beakers.

Using a wax pencil, label the beakers "15% sucrose cell" and "30% sucrose cell". Fill each beaker with 200 ml water.

3) By gently massaging one end of the dialysis tube with your fingers, open it.

Clamp the other end of the tube shut by first folding that end in half **sideways** and then applying a plastic clamp to the sideways fold. Your instructor has prepared a demonstration clamped dialysis tube to help you with this step.

Carefully fill the tube with 25 ml of 30% sucrose solution. Fold the open end of the dialysis tube in half (expelling as much air as possible) then clamp it shut. **Leave empty room for the cell to expand.** This dialysis tube represents a "cell" with a solute concentration of 30 grams per 100 ml. If done properly, your tube should not leak and should look the same as the instructor's demonstration tube. Using paper towels, gently pat dry the "cell". Try to remove all drops of water from the surface of the tube and from the plastic clamps.

Tare (zero) an electronic balance. Place the "cell" on the balance and record its mass in results data table A on page 8 of this handout.

Place the cell in the "30% Sucrose cell" beaker of water. Be sure that the cell is fully submerged under the water.

4) Get a second dialysis tube from the front desk. Repeat step 3 with the new dialysis tube, but fill the tube with 15% sucrose solution instead of the 30% sucrose solution.

5) Set a timer and let the two dialysis tube “cells” soak in the water beakers for 30 minutes.

While the dialysis tubes are soaking in the water beakers, do activities d, e, and f (the diffusion activities).

6) After 30 minutes of soaking, remove the 15% sucrose cell. Use paper towels to gently pat dry the cell. Again, try to remove all drops of water from the surface of the tube and from the plastic clamps.

Tare (zero) an electronic balance. Place the cell on the balance and record its mass in results data table A.

7) Repeat step 6 with the 30% sucrose cell.

When you have entered your data into table A, show your results to your instructor. Your instructor will then give you the results for the same osmosis experiment but with 15% albumin protein inside a "cell" instead of 15% sucrose inside the "cell". Copy those albumin results into data table A.

8) Answer review questions 12 and 13, then in the space below write a hypothesis about the effects of solute concentration on osmosis rate. Your hypothesis should explain all your results. **Show your instructor your hypothesis and your answers to review questions 12 and 13.**

9) Clean up: Save the dialysis clips. The membranes and solutions can be disposed of in the garbage and the sink.

c) The effect of solute type on osmosis rate

Do this activity only if you are in one of the Left-side groups of the lab. If you are a Right-side lab group, do activity (b) instead. But both groups should give their data to the instructor when the activity is finished.

All cells have solutes inside. Suppose a cell was put into pure water (which has a lower solute concentration than the cell). The principle of osmosis states that water will move into the cell, but is the rate of water movement affected by the type of solute inside the cell? In other words, if two cells had the same grams of solutes inside as one another but each cell had a different type of solute inside than the other cell, would that cause the cells to gain different amounts of water than each other? To test this question, you will make two “cells” out of dialysis tubing and fill them with different solutes. The two cells will have the same grams of solutes inside (15% concentration of solutes, which means 15 grams of solutes per 100 mL) but the solute in one cell will be sucrose (a small disaccharide molecule. Molecular weight = 342 grams per mole) and the solute in the

other cell will be albumin (a large protein molecule. Molecular weight = 67,000 grams per mole).

1) On the front desk is a supply of dialysis tubes that are soaking in a beaker of water. Obtain one piece of dialysis tubing from the beaker. Also obtain four plastic clamps and two empty 300 ml beakers.

Using a wax pencil, label the beakers "15% sucrose cell" and "15% albumin cell". Fill each beaker with 200 ml water.

3) By gently massaging one end of the dialysis tube with your fingers, open it.

Clamp the other end of the tube shut by first folding that end in half **sideways** and then applying a plastic clamp to the sideways fold. Your instructor has prepared a demonstration clamped dialysis tube to help you with this step.

Carefully fill the tube with 25 ml of 15% sucrose solution. Fold the open end of the dialysis tube in half (expelling as much air as possible) then clamp it shut. **Leave empty room for the cell to expand.** This dialysis tube represents a "cell" with a solute concentration of 15 grams per 100 ml. If done properly, your tube should not leak and should look the same as the instructor's demonstration tube. Using paper towels, gently pat dry the "cell". Try to remove all drops of water from the surface of the tube and from the plastic clamps.

Tare (zero) an electronic balance. Place the "cell" on the balance and record its mass in results data table A on page 8.

Place the cell in the "15% Sucrose cell" beaker of water. Be sure that the cell is fully submerged under the water.

4) Get a second dialysis tube from the front desk. Repeat step 3 with the new dialysis tube, but fill the tube with 15% albumin solution instead of the 15% sucrose solution.

5) Set a timer and let the two dialysis tube "cells" soak in the water beakers for 30 minutes.

While the dialysis tubes are soaking in the water beakers, do activities d, e, and f (the diffusion activities).

6) After 30 minutes of soaking in water, remove the 15% albumin cell. Use paper towels to gently pat dry the cell. Again, try to remove all drops of water from the surface of the tube and from the plastic clamps.

Tare (zero) an electronic balance. Place the cell on the balance and record its mass in results data table A.

7) Repeat step 6 with the 15% sucrose cell.

When you have entered your results in data table A, show your results to your instructor. Your instructor will then give you the results for the same osmosis experiment but with 30% sucrose inside the "cell" instead of 15% sucrose inside the "cell". Copy those sucrose results into results data table A.

8) Answer review questions 12 and 13, then in the space below write a hypothesis about the effects of solute concentration on osmosis rate. Your hypothesis should explain all your results. **Show your instructor your hypothesis and your answers to review questions 12 and 13.**

9) Clean up: Save the dialysis clips. The membranes and solutions can be disposed of in the garbage and the sink.

d) Introduction to diffusion

Imagine putting a drop of ink into a glass of water. At first the ink drop would be concentrated in just a small area of the water, but as time passed the ink would spread out. This is an example of diffusion. Diffusion is when solute molecules move from areas where they are at higher concentration to areas where they are at low concentration.

Diffusion is vital to many life processes. Your nervous system, for example, relies on diffusion of solutes to pass signals from one nerve cell to another. Since the body fluids of life forms here on earth are mostly water, diffusion of the solutes in organisms is usually through aqueous (watery) body liquids.

What causes diffusion to occur? All substances contain energy in the form of heat. Even something that feels very cold to the touch contains at least a little heat energy. The heat energy is really the motion of the substances' molecules. The heat causes the molecules to move in random directions, bouncing off each other like billiard balls at the start of a game of pool. Just as the random motion of the billiard balls tends to spread them out from each other on the pool table, the random heat movement tends to spread the molecules out from each other.

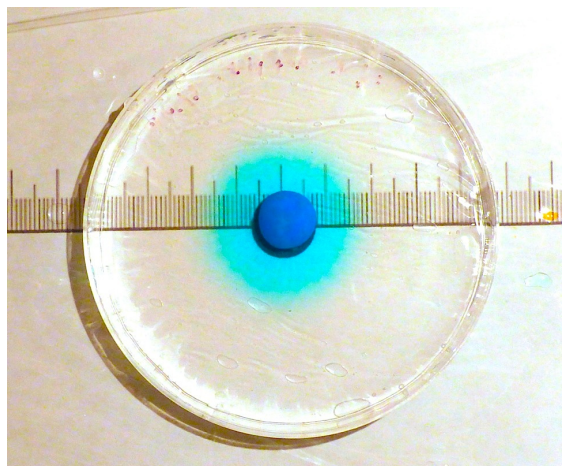
In today's lab the solutes that you will use for diffusion are the food colors dyes in the coating of M&M© candies. You will investigate how the rate (speed) of a solute's diffusion is affected by temperature and by the molecular weight of the solute.

e) The effect of a solute's molecular weight on its diffusion rate

Does the mass (heaviness) of a molecule affect how fast it diffuses, or do all molecules diffuse at the same rate? In this activity, you will find out by comparing the diffusion rates of two dye molecules in M&Ms: A blue food color dye and an orange food color dye. The blue food color molecule has a mass (molecular weight) of 793 grams per mole. The main dye in orange M&Ms has a molecular weight of 534 grams per mole. In other words, the blue dye molecules have a larger mass than the orange dye molecules.

- 1) Obtain a plastic petri dish plate and a white diffusion card. Place the petri dish on top of the card such that the center circle on the card is under the center of the petri dish.
- 2) Get the beaker of room temperature water from the front desk and pour water into the petri dish until the dish is almost full.
- 3) When the water has stopped moving, get a blue M&M. Place the M&M onto the circle at the center of the petri dish. Start a 1 minute timer as soon as the M&M enters the water.
- 4) After one minute, measure the diameter of the dye's diffusion circle using the lines on the card. Each tiny line is 1 millimeter. The largest lines are 10 millimeters apart.

As an example, the diameter of the dye diffusion circle below is about 32 millimeters.



The rate of the dye's diffusion per minute is the same as the diameter of the diffusion circle that you measured. Record the dye's rate of diffusion (the diameter of its diffusion circle) in data table B.

- 5) Dump out the water and repeat steps 2 - 4 but use an orange M&M instead of a blue M&M.

f) The effect of temperature on a solute's diffusion rate

Does temperature affect a solute's rate of diffusion? In this activity, you will compare the diffusion rate of the blue M&M dye at different temperatures.

- 1) Obtain a plastic petri dish plate and a white diffusion card. Place the petri dish on top of the card such that the center circle on the card is under the center of the petri dish.
- 2) Get the beaker of ice cold temperature water from the front desk and pour water into the petri dish until the dish is almost full. Ice water is 0 degrees celsius.
- 3) When the water has stopped moving, get a blue M&M. Place the M&M onto the circle at the center of the petri dish. Start a 1 minute timer as soon as the M&M enters the water.
- 4) After one minute, measure the diameter of the dye's diffusion circle using the lines on the card. Each tiny line is 1 millimeter. The largest lines are 10 millimeters apart. The diameter of the diffusion circle is the distance that the dye diffused in one minute. Record the distance of the dye's diffusion in data table C.
- 5) Dump out the water and repeat steps 2 - 4 with another blue M&M but this time use boiling hot water instead of the ice water (your instructor will pour the boiling water for you). Boiling water is 100 degrees celsius.

g) Results section:

Data Table A:

<u>Cell:</u>	<u>Mass (grams) before osmosis</u>	<u>Mass (grams) after osmosis</u>	<u>Mass increase</u>
30% sucrose	_____	_____	_____
15% sucrose	_____	_____	_____
15% albumin	_____	_____	_____

Data Table B:



<u>Dye color</u>	<u>Dye molecular weight</u>	<u>Dye diffusion rate (in mm per minute)</u>
<u>Blue</u>	_____	_____
<u>Orange</u>	_____	_____

Data Table C:

<u>Temperature (in degrees celsius)</u>	<u>Dye diffusion rate (in mm per minute)</u>
<u>0 (ice water temperature)</u>	_____
<u>20 (room temperature)</u>	_____*
<u>100 (boiling temperature)</u>	_____

* Use the same rate as your blue dye result from data table B.

h) Review questions

- 1) Define diffusion as it was defined in this handout, and explain at a molecular level what causes it to occur.
- 2) State the relationship between a solute molecular weight and its diffusion rate. Explain, at a molecular level, why your answer is true.
- 3) State the relationship between temperature and diffusion rate. Explain, at a molecular level, why your answer is true.
- 4) Everything else being equal, on a cold day warm-blooded animals will have faster reflexes than cold-animals. Why?
- 5) Define osmosis as it was defined in this handout.
- 6) Define semi-permeable membrane as it was defined in this handout.
- 7) In the blank after each situation described below,
 - ...draw a shrivled cell if the situation would cause a cell to lose water 
 - ...draw a bursting cell if the situation would cause a cell to gain water 
 - ...draw a round cell situation would not cause a cell to gain or lose water. ○
 - a) The cell is in a hypertonic solution: _____
 - b) The cell is in pure water: _____
 - c) The cell is in an isotonic solution: _____
 - d) The cell is in water from the dead sea (very very salty): _____
 - e) The cell is in a hypotonic solution: _____
 - f) There are more solutes outside the cell than inside the cell: _____
- 8) Visually, a dialysis tube appears to be a solid barrier. How are the water molecules able to pass through it? Why aren't other molecules (like sucrose and albumin) able to pass through as easily as water?
- 9) If the sucrose and albumin were able to easily pass through the dialysis membrane, would the cells have gained any water by osmosis? Why or why not?

10) How did you measure how much osmosis occurred in your dialysis “cells”?

11) Before weighing the dialysis tube, you were instructed to dry them with a paper towel. Why was this step necessary? In what way would it have affected the apparent rate of osmosis if you had forgotten to dry the tube?

12) Which cell, 30% sucrose or 15% sucrose, gained more water by osmosis? Explain why.

13) Which cell, 15% albumin or 15% sucrose, gained more water by osmosis? Explain why.

i) Review question answers

1) Diffusion is the movement of solutes from areas of their higher concentration to areas of their lower concentration. It is caused by the kinetic energy of heat causing the solute molecules to move in random directions.

2) The higher the molecule weight of a solute molecule, the lower its diffusion rate (and *vice versa*). The reason for this is that heavier objects have more inertia (resistance to movement) than lighter objects. So the same amount of heat energy (in other words, the same temperature) will cause less movement of heavier molecules and more movement of lighter molecules.

3) The higher the temperature, the more kinetic energy of heat is present. Because heat kinetic energy is what causes diffusion, a higher temperature will cause faster diffusion.

4) Many processes in the body require diffusion of solutes. For example, muscle contraction requires diffusion of calcium ions into muscle cells. Because the rate of diffusion is affected by temperature, cold blooded animals will have slower reflexes (slower muscle contraction) than warm blooded animals on cold days.


5) Osmosis is the movement of water across a semi-permeable membrane towards whichever side of the membrane has the highest solute concentration.

6) A semi-permeable membrane is a membrane that allows water molecules to pass through but not solute molecules.

7)

a) 

b) 

c) 

d) 

e) 

f) 

8) The dialysis membrane has millions of microscopic holes. Water molecules are small enough to easily pass through these holes, but larger solute molecules (such as sucrose and albumin) are too big to pass through the holes.

9) There would be no significant water gain by the cells if the sucrose and albumin solutes were able to easily pass through the dialysis membrane. If those solutes were able to pass through the membrane, they would quickly diffuse out of the cell into the surrounding water, until the solutes were at the same concentration inside the cell as outside the cell. That would mean that the cells would soon be in an isotonic solution, and no net osmosis occurs when cells are in an isotonic solution.

10) The weight gain of the cell was how the amount of osmosis of each cell was measured. The more osmosis, the more water entered the cell, and so the more weight the cell gained.

11) Drying the cell removed water drops clinging to the outside of the cell. The goal of our experiment was to measure the amount of osmosis that occurred for each cell, and the amount of osmosis was given by the weight of water molecules that entered the cell (not the weight of water molecules on the outside of the cell). So removing the water on the outside of the cell allowed us to have a more accurate measurement of the osmosis that occurred. If we had not removed the water on the outside, we would have incorrectly included that extra water in our osmosis weight change calculation, so we would have incorrectly thought that a higher rate of osmosis had occurred than had actually occurred.

12) The 30% sucrose cell gained more water by osmosis than the 15% sucrose cell. Osmosis occurs because solutes attract water molecules. The 30% sucrose cell had twice the amount of solute molecules than the 15% sucrose cell, so roughly twice as much osmosis occurred in the 30% sucrose cell.

13) The 15% sucrose cell gained more water by osmosis than the 15% albumin cell. Osmosis occurs because solute molecules attract water molecules. It is the number of solute molecules present that determines how much osmosis occurs, not the total weight of solute molecules. The 15% sucrose cell and the 15% albumin cell had the same percentage concentration (which means that the total weight of solute inside those cells was the same, because percentage concentration units are based on the total weight of the solute present). Simplifying slightly, you can think of the 15% concentration of the cells as meaning that each cell had 15 grams of solute inside. But individual sucrose molecules are much smaller and lighter than albumin molecules, so it takes more sucrose molecules to total up to 15 grams than it does albumin molecules*. Therefore, the 15% sucrose cell had more molecules inside than the 15% albumin cell. That is why the 15% sucrose cell gained more water by osmosis.

*As an analogy for this concept, imagine two boxes. One box contains 100 pounds of ping-pong balls and the other box contains 100 pounds of bowling balls. 100 pounds of

ping-pong balls is about 16,800 balls. But 100 pounds of bowling balls is only about 7 balls. So two groups of objects can have the same total weight as each other but this does not mean that they have the same number of objects. The group with the lighter objects must have more objects to add up to the same weight as the group with the heavier objects.