Lab 4
Diffusion, Osmosis, and Tonicity

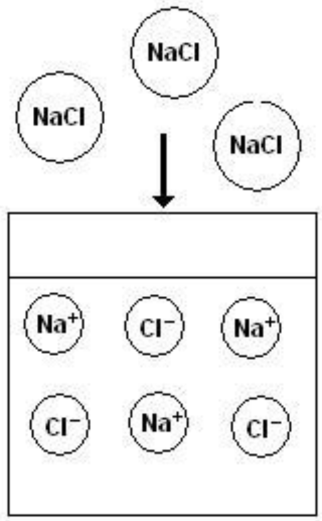
OBJECTIVES:

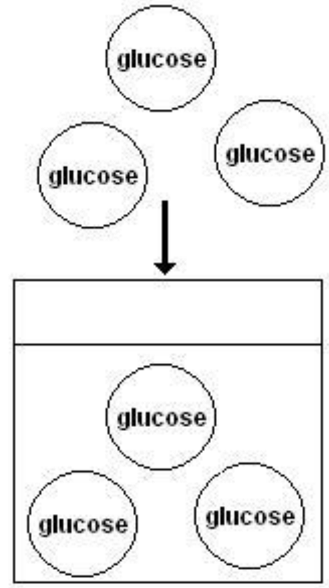
1. Be able to define molarity, osmolarity, tonicity, isotonic, hypotonic, hypertonic, crenate, lyse, concentration gradient, diffusion, osmosis, and osmotic pressure and hydrostatic pressure.
2. Understand the various ways that concentration can be expressed.
3. Be able to predict the osmolarity of a solution.
4. Know the factors that affect the rate of diffusion (and osmosis) and understand why they have the effects they do.
5. Be able to predict and explain the effect of placing cells in solutions of varying tonicities.
6. Be able to identify and use the following piece of laboratory equipment:
    a. Dialysis tubing

CONCENTRATIONS:

Remember that the concentration of a solution can be expressed in many ways. Molarity is one measure of concentration. A mole of any substance contains 6.02×10^{23} atoms or molecules. One mole of NaCl contains 6.02×10^{23} NaCl formula units; likewise, one mole of glucose contains 6.02×10^{23} glucose molecules. A mole is the number of grams of a formula that is equal to its atomic or molecular mass. For NaCl, the formula mass is equal to the mass of one Na atom + the mass of one chlorine atom, so it is 23 + 35.5 or 58.5. By definition, 58.5g of NaCl equals 1 mole. A one molar (1M) solution of NaCl contains 1 mole of NaCl per liter of solution.

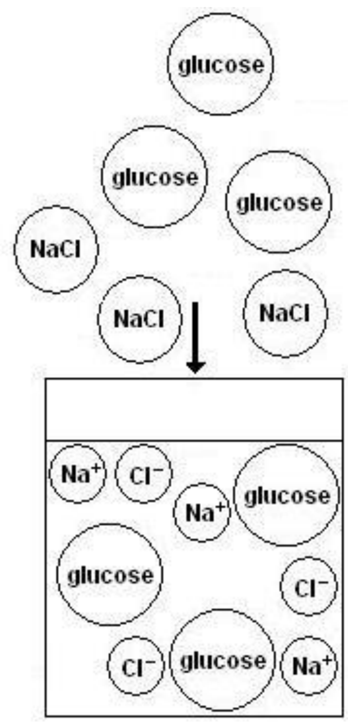
What about glucose? The chemical formula for glucose is $C_6H_{12}O_6$. Each carbon atom has a mass of 12 amu, each hydrogen has a mass of 1 amu, and each oxygen has a mass of 16 amu. (6 x 12) + (12 x 1) + (6 x 16) = 72 + 12 + 96 = 180. Therefore, I mole of glucose has a mass of 180g. A one molar (1M) solution of glucose would contain 1 mole, or 180g, of glucose per liter of solution.





Although a 1M solution of NaCl contains the same number of NaCl units as a 1M solution of glucose, they behave differently. Remember that NaCl is held together by ionic bonds. In solution, the NaCl crystals separate (dissociate) into their component ions, so the solution would contain 1 mole of Na^+ ions and 1 mole of Cl^- ions. Glucose, on the other hand, does not separate (dissociate) in solution. A 1M solution of NaCl, therefore, contains twice as many solute particles as a 1M glucose solution.

What if we add a mole of glucose to the NaCl solution? Now we have one mole each of Na^+, Cl^-, and glucose in the beaker. To account for this, we can turn to osmolarity, another measure of concentration. Osmolarity gives the *total* number of solute particles in the solution. The solution of glucose and NaCl just created is a 3 osmolar solution.



The solute concentration of blood plasma is approximately 0.3 osmoles per liter or 300 milliosmoles/liter. Tonicity is a comparison between the osmolarity of a solution and the osmolarity of blood plasma. A hypertonic solution contains a higher total solute concentration than does blood plasma, or > 300 mosm/l. It has a higher osmotic pressure than plasma.

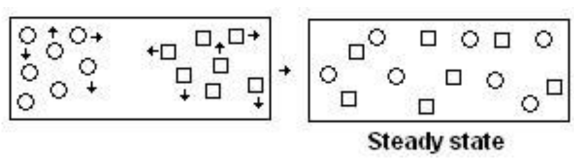
An isotonic solution contains the same total solute concentration as blood plasma, or = 300 mosm/l. It has the same osmotic pressure as plasma. A hypotonic solution contains a lower total solute concentration than blood plasma, or < 300 mosm/l. It has a lower osmotic pressure than plasma.

Remember that concentration can be expressed in various ways. Sometimes, the concentration of a solution will be expressed as the number of grams of solute per volume of solution. For example, you could measure out 3.5g of NaCl and add enough water to create a deciliter of solution; the concentration of this NaCl solution would be 3.5g/dl. We know that concentration can also be expressed as a percentage. To make a 10% NaCl solution, you could measure out 10g of NaCl and add enough water to create 100 ml of solution. Think about making a 10% solution of glucose. You would measure out 10g of glucose and add enough water to create 100ml of solution.

- *Knowing what you do about the formula mass of NaCl and the molecular mass of glucose, would 10.g of glucose contain more or fewer particles than would be found in 10.g of NaCl? Please show all your work and express your answer with the appropriate number of significant figures and in scientific notation.*

DIFFUSION:

Diffusion is important in physiology because it is the way many substances move through the cell membrane. O_2 and CO_2 diffuse through the lipid bilayer, ions diffuse through protein channels, and glucose uses a membrane carrier protein to move via facilitated diffusion. Very simply, diffusion occurs because molecules are in motion. Because molecules are moving randomly, they tend to have net movement from areas of higher concentration to areas of lower concentration until they are uniformly distributed. At that time, molecules are still moving, but no further net movement is taking place; a steady state has been reached.

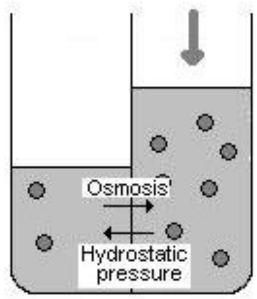


Because it depends on the kinetic energy of the molecules themselves, diffusion does not require the input of additional energy. It is driven by concentration gradients or differences in concentrations. Several factors can affect the rate of diffusion, including the magnitude of the concentration gradient, the formula mass of the substance, and temperature. A steeper concentration gradient increases the rate of diffusion. Particles with less mass move faster than particles with greater mass. In addition, particles move faster at high temperatures than at low temperatures. Therefore, you can predict how temperature and particle size will affect the rate of diffusion.

When diffusion is occurring through a membrane, the surface area, permeability, and thickness of the membrane affect the rate. Increasing surface area, increasing permeability, and decreasing the thickness of the membrane will all increase the rate of diffusion.

OSMOSIS:

Osmosis is a special case of diffusion. Osmosis refers to the movement of water through a semipermeable membrane from an area of higher water concentration (lower total solute concentration) to an area of lower water concentration (higher total solute concentration). Osmosis depends on the difference in osmolarity between solutions on opposite sides of a semipermeable membrane. The solution with higher osmolarity has a higher osmotic pressure. Water will move into the solution with higher osmotic pressure, and osmosis will continue until a steady state is reached or until the osmotic pressures balance. Remember that osmotic pressure can be opposed by the development of hydrostatic pressure, the pressure exerted by a column of fluid.



TONICITY:

An isotonic solution has the same osmolarity as blood plasma. A cell placed in an isotonic solution does not have a water concentration gradient across its membrane; intracellular solute concentration equals the extracellular solute concentration. There will be no net movement of water across the cell membrane, and the cell will not change in volume. If a cell were placed in a hypertonic or hypotonic solution, concentration gradients for water exist. Osmosis will occur, and the cell will either lose water to the solution (decrease in volume) or gain water from the solution (increase in volume or even lyse).

- *What effect would a hypertonic solution have on cell size?*
- *What effect would a hypotonic solution have?*

NEW LABORATORY EQUIPMENT:

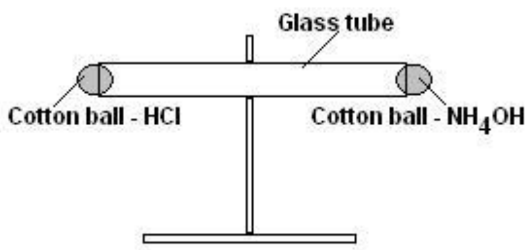
Dialysis tubing is made of cellulose or cellophane and functions as a semipermeable or selectively permeable membrane. Small particles can pass through the membrane via diffusion or osmosis, but larger particles cannot pass through the membrane's pores.

CAUTION:

This lab involves working with blood, a potentially hazardous body fluid. Remember to wear GLOVES and SAFETY GLASSES if you are handling a blood sample that is not your own. Follow all cleanup instructions so that other students, your instructor, and cleaning personnel do not risk exposure to bloodborne pathogens. If you have any questions about how to proceed, ask your instructor.

PROCEDURE:

1. Effect of Molecular Mass on the Rate of Diffusion (Demonstration)



Under the lab's ventilation hood, you will see a setup like the one pictured above. Your instructor will dip one cotton ball into a concentrated solution of hydrochloric acid (HCl) and one into a concentrated solution of ammonium hydroxide (NH_4OH). The cotton balls will be inserted into the ends of the glass tube at the same time. Cl^- ions and NH_4^+ ions will diffuse toward the center of the tube. When the two ions meet, they will react and form ammonium chloride, a white solid that will be visible in the tube. The reaction is as follows:

$$Cl^-_{(gas)} + NH_4^+{}_{(gas)} \longrightarrow NH_4Cl_{(white\ solid)}$$

Given the following information about atomic masses, calculate the molar mass of Cl^- and of NH_4^+.

Element	Atomic Mass (amu)
H	1
N	14
Cl	35.5

- *Which gas would be expected to diffuse more quickly? Why?*

Measure the distance traveled by each gas (from the inner edge of the cotton to the area where the white precipitate can be seen) in centimeters.

	Cl^-	NH_4^+
Molar mass		

Distance traveled		

- *Do your results support your prediction? If not, what do you think might have happened?*

2. Effect of Temperature on the Rate of Diffusion (Demonstration)

On the front bench, you will see 2 beakers of water. One beaker contains hot water and the other contains cold water. A red Atomic Fireball candy or a crystal of dye will be placed in each beaker. You will observe the 2 beakers every 1 minutes and record your observations.

- *In which beaker would you expect the diffusion to occur faster? Why?*

Time (min)	Hot Water	Cold Water
0		
1		
2		
3		
4		
5		

- *Did the results support your prediction? If not, what do you think might have happened?*

3. Osmosis – Dialysis Tubing (To be done by each group)

In this experiment, you will investigate the effect of solute concentration on the rate of osmosis. Bags made of dialysis tubing will be filled with either distilled water or varying concentrations of sucrose solutions. The pores of the dialysis tubing are permeable to water. Sucrose is a disaccharide and is not able to pass through the pores of the dialysis tubing. Why?

1. Cut 4 strips of dialysis tubing; each strip should be approximately 15 cm long.

2. Soak the strips in tap water for 5 minutes, and then roll them between your thumb and index finger to open the tubing.
3. Fold over one end of a strip, and apply a clip to close the end of the tube.
4. Repeat with the remaining 3 strips of tubing so that you have a total of 4 “bags”.
5. Label four beakers 1-4. Fill each beaker about half full of distilled water.
6. Fill bag 1 as indicated in the table below. Fold over the open end of the tubing and secure it with a clip.
7. Find the mass of the bag to the nearest tenth of a gram (one decimal place) and record it in the table (0 minutes).
8. Repeat steps 6 and 7 for bags 2-4.
9. Place each bag in its beaker.
10. Every 15 minutes, remove each bag from its beaker, dry it carefully on paper towel, and find its mass. Record your results in the table.

	Bag 1	Bag 2	Bag 3	Bag 4
Solution	15ml distilled water	15ml 20% sucrose	15ml 40% sucrose	15ml 60% sucrose

Time (Minutes)	Mass of Bag (grams)							
	Bag 1 (distilled water)		Bag 2 (20% sucrose)		Bag 3 (40% sucrose)		Bag 4 (60% sucrose)	
0								
15								
30								
45								
60								

Calculate the rate of osmosis and record in chart below.

Rate is a measurement of change over time.

To determine the rate for each bag, use the following formula:

[Final mass (g) – initial mass (g)] / total time = Rate of Osmosis g/min

	Bag 1 (distilled water)	Bag 2 (20% sucrose)	Bag 3 (40% sucrose)	Bag 4 (60% sucrose)
Rate of Osmosis				

Create a graph showing the rate of osmosis for each concentration of sucrose. Review the instructions for making a graph used in Week 2.

- *Which concentration showed the fastest rate of osmosis?*
- *Is there a clear relationship between rate of osmosis and solute concentration?*

- *Explain your results at a molecular level. How does solute concentration effect the movement of water molecules during osmosis?*

4. Tonicity – (To be done by each group)

One group member will obtain a blood sample via fingerstick following the directions given by your instructor. The red blood cells will be exposed to three solutions of sodium chloride: a hypertonic solution of 3.0g/dl, an isotonic solution of 0.90g/dl, and a hypotonic solution of 0.40g/dl. You will observe the cells under the light microscope to determine the effects of the NaCl solutions on cell volume.

1. Label the 3 covered, plastic tubes A, B, and C.
2. Using a micropipette, dispense 1.0 ml of solution A into tube A, 1.0ml of solution B into tube B, and 1.0 ml of solution C into tube C.
3. Place one drop of blood into each plastic tube. Close the cover of each tube and gently mix the blood drop with the NaCl solution.
4. Using a disposable plastic pipette, transfer one drop of the mixture in tube A to a glass slide labeled "A." Cover the sample with a coverslip.
5. Examine slide A under the microscope using high power. Note the appearance (shape and size) of the red blood cells and record it in your table. (Do not dispose of slide A until you have a chance to look at all three slides.)
6. Repeat steps 4 and 5 for tubes B and C.
7. Fill in the chart using your observations (RBC appearance) , understanding (Tonicity) and the information provided (NaCl solutions).

Tube #	NaCl Solution	RBC Appearance	Tonicity	Concentration of NaCl Solution
A	A			
B	B			
C	C			

In your own words, describe what is happening when a cell is placed in the hypertonic, isotonic and hypotonic solutions. Explain why water is moving in the direction it moves and how that changes cell shape. Be sure to include the relationship between solute concentration and water molecule concentration.