Diffusion and Osmosis

a) 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.

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 this lab you will demonstrate diffusion and investigate how the rate (speed) of a solute's diffusion is affected by temperature, solute concentration, and the molecular weight of the solute.

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. In today's lab, however, you will demonstrate diffusion of solutes through a gel-like substance called agar. The agar (which is made from seaweed) is mostly water, but its solidity will make it easier to measure diffusion of solutes.

b) The effect of molecular weight on diffusion rate

Does the mass (heaviness) of a molecule affect how fast it diffuses, or are all molecules small enough that they diffuse at the same rate? In this activity, you will compare the diffusion rates of two dye molecules: Food color blue and methylene blue. The food color blue has a lower molecular weight than methylene blue.

1) Obtain two agar plates. Use a cut 3ml pipette to make a well in the center of both plates. It helps to squeeze the pipette bulb before inserting it into the agar, then to release the bulb to suck out the agar plug from the well.

Label one plate "FC room temperature" and label the other plate "MB room temperature"

2) To the well in the FC plate, add 3 drops of blue food coloring. Be careful not to spill any food coloring on the agar outside the well.

To the MB plate, add 3 drops of methylene blue dye. Again, be careful not to spill any dye on the agar outside the well.

3) Put both plates on your desk top. Write down the current time in the margin of this page, because in one hour, you will check the diameter of the dye diffusion in each plate.

4) In after one hour, obtain a ruler. Measure the diffusion radius of each dye. Measure from the center of the well to the closest diffusion edge. Record the results in data table A.

c) The effect of temperature on diffusion rate

Does temperature affect the speed of diffusion? In this activity, you will compare the diffusion rate of the blue food color at two different temperatures.

1) Obtain an agar plate from the 55 degree incubator. Use a cut 3ml pipette to make a well in the center of the plate. It helps to squeeze the pipette bulb before inserting it into the agar, then to release the bulb to suck out the agar plug from the well. Label that plate “FC 55 degrees”.

2) Add 3 drops of blue food coloring. Be careful not to spill any food coloring on the agar outside the well.

3) Return the “55 degree” plate to the 55 degree incubator. Write down the current time in the margin of this page.

4) Repeat steps 1 – 3, but with a cold plate from the refrigerator in the side room. Label this plate “FC 4 degrees”. After adding the food color to this plate, return it to the refrigerator.

5) In after one hour, obtain a ruler. Measure the diffusion radius of each dye. Measure from the center of the well to the closest diffusion edge. Record the results in data table A.

d) The effect of solute concentration on diffusion rate

Will the concentration of a solute change its rate of diffusion? In this activity, you will compare the diffusion rate of two different concentrations of blue food color.

1) Obtain two small test tubes. Using a wax pencil, label one tube 1:10 and the other tube 1:100. To the 1:10 test tube, add nine drops water and one drop blue food color. To the 1:100 test tube, add 1 drop of the 1:10 dilution and 9 drops of water.

2) Obtain 2 agar plates. Use the cut 3ml pipette to make a well in the center of both plates.

Label one plate “FC 1:10” and one plate “FC 1:100 ”.

2) To the “FC 1:10” plate, add 3 drops of the 1:10 dilution of food color. To the “FC 1:100” plate. add 3 drops of the 1:100 dilution of food color.

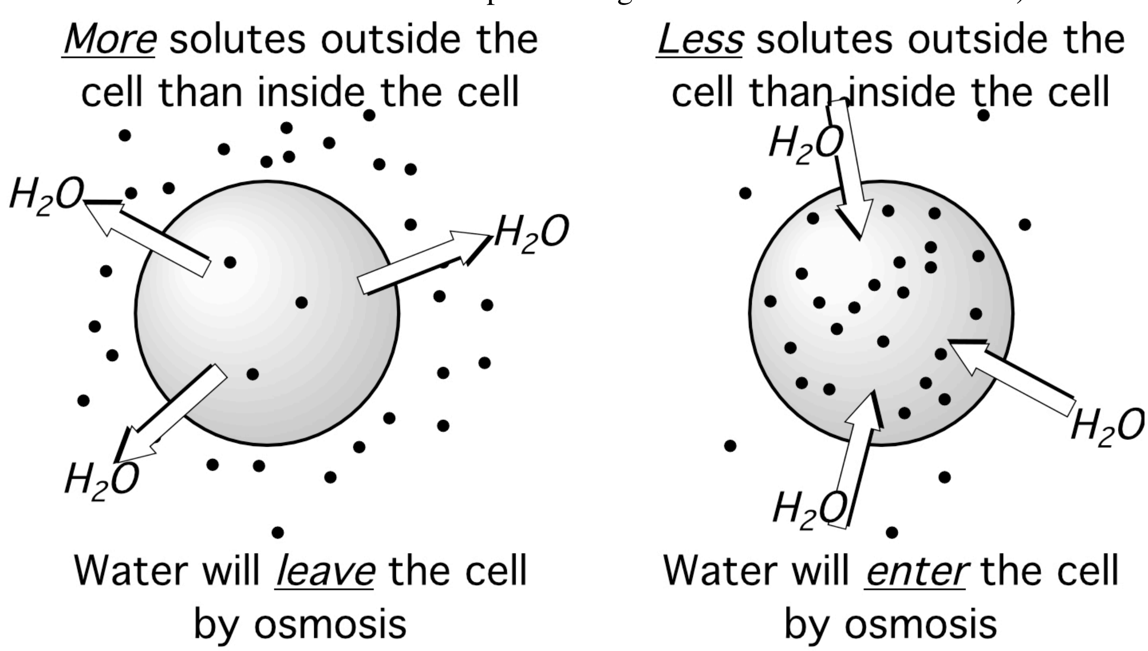
3) Put both plates on your desk. Write down the current time in the margin of this page.

4) In after one hour, obtain a ruler. Measure the diffusion radius of each dye. Measure from the center of the well to the closest diffusion edge. Record the results in data table A. **Show your instructor your results when done with this activity.**

5) Clean up: The plates can be put into the trash. The small glass test tubes used for the dilution should go into the glass waste (not the trash).

e) 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 to 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).

f) The effect of solute concentration on osmosis rate

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 cell's solute concentration, would it gain more water? To test this question, you will make "cells" out of dialysis tubing and fill them with solutes. The solutes will either be 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) Obtain three 5.5 inch sections of dialysis tubing, six plastic clamps for the tubes, and three 300 ml beakers.

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

2) Place all three dialysis tubes into the "Water" beaker. Let them soak for 5 minutes.

3) After 5 minutes, remove one dialysis tube. By gently massaging one end of the 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 table B.

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

4) Repeat step 3 with another dialysis tube, but use the 15% sucrose solution.

5) Repeat step 3 with another dialysis tube, but use the 15% albumin solution.

6) Let the three dialysis tube "cells" remain in the water for an hour.

7) After one hour, remove the 30% 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 table B.

8) Repeat step 7 with the 15% sucrose cell and the 15% albumin cell.

9) Answer review questions 15 and 16, 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 15 and 16.**

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

g) Results section:

Data Table A:

Plate:	Radius (millimeters):
Food color, room temperature	_________
Methyl blue, room temperature	_________
Food color, 4 degrees C	_________
Food color, room temperature*	_________
Food color, 55 degrees C	_________
Food color, no dilution*	_________
Food color, 1:10 dilution	_________
Food color, 1:100 dilution	_________

* Use the same radius as your first blue food color result.

Data Table B:

Cell:	Mass (grams) before osmosis	Mass (grams) after osmosis	Mass increase
30% sucrose	______	______	______
15% sucrose	______	______	______
15% albumin	______	______	______

g) Review questions

1) Define diffusion and explain at a molecular level what causes it to occur.

2) State the relationship between solute concentration and diffusion rate.

3) State the relationship between temperature and diffusion rate. Explain, at a molecular level, why your answer is true.

4) Someone has hypothesized that for every 10ºC increase in temperature, molecules double in speed. Does your temperature data support or refute this hypothesis? Why or why not?

5) In the blood vessels of the lungs oxygen diffuses from the air into the blood. The blood then circulates to all parts of your body, so that your cells can receive oxygen. You saw today that solutes can diffuse through liquids even if those liquids are not moving (such as in your agar dishes) So why does your body bother to circulate the blood? Why not just let the oxygen diffuse from the lungs to the cells?

6) State the relationship between a solute molecular weight and its diffusion rate. Explain, at a molecular level, why your answer is true.

7) Everything else being equal, on a cold day warm-blooded animals will have faster reflexes than cold-animals. Why?

8) Define osmosis. Give the full and complete definition.

9) Define semi-permeable membrane:

10) 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: ______

11) Visually, the 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?

12) 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?

13) How did you measure how much osmosis occurred in your dialysis "cells"?

14) 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?

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

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