

Topic 7. Diffusion

Introduction: This exercise explores the physical phenomenon of **diffusion** and specifically the special case of diffusion of water through a differentially permeable membrane. This process is **osmosis**. We will also observe the rates of diffusion into dry seeds as their cell walls **imbibe** water. A secondary goal is to develop cooperative skills working in a group.

Diffusion: Diffusion is simply the net movement of substances in a gas or a liquid from areas of higher concentration to areas of lower concentration. The movement of substances into and out of cells (and, indeed, into and out of the bodies of organisms) is accomplished largely by diffusion.

The Cell Membrane and Osmosis: Cells are islands of order where the processes of life are sequestered from the randomness of the nonliving environment. The smallest thing that is truly alive is a cell. The structure that serves as the gatekeeper between the living and nonliving world is the cell membrane. The cell membrane is a **differentially permeable membrane**. As the name denotes, it is differentially permeable to various substances. Water, for example, freely crosses this boundary, but sodium and potassium ions will not pass through at all unless special portals are present to allow transport. The membrane we will use in these exercises is dialysis tubing (a nonliving sheet of cellophane). However, the principles you will observe here are essentially the same as those for living membranes.

Osmosis is the diffusion of water across a differentially permeable membrane. Because cells exist in aqueous environments, control of osmosis is critical to their survival. A cell either needs to maintain equal concentrations of water on both sides of its plasma membrane, or the cell needs some mechanism for maintaining a difference. There are three possible conditions in regards to the concentration of water in the cell relative to its environment:

- 1. Isotonic** - the solute concentration is the same on both sides of the cell membrane. This is the condition of the cells in the human body. The fluids in our body (blood and plasma) are isotonic to the cells in our body.
- 2. Hypotonic** - the solute concentration is lower outside of the cell than inside. Under these circumstances a cell is at risk of bursting. Some protozoans have contractile vacuoles that expel excess water to prevent bursting. If blood cells are placed in distilled water they will burst. Many protists, all the fungi, and all plant cells regulate the inflow of water under these conditions by means of the cell wall. While the membrane is in contact with the cell wall the wall will provide a static force that equals the force generated by the tendency of water to move into the cell. This not only prevents the cell from bursting, but, in the case of herbaceous plants, also provides support like that provided by an inner tube in a tire.

3. Hypertonic - The solute concentration is greater outside the cell than inside. A cell under these conditions will tend to shrink. This often represents an abnormal, stressful condition (the cells of a wilting plant, dehydration due to drinking sea water etc.).

Diffusion and imbibition: Another physical phenomenon linked to diffusion is imbibition. Imbibition is the diffusion of water into a dehydrated substrate having an affinity for water. Examples of imbibition include the expansion of seeds, doors and sponges when they absorb water. Osmosis is not involved in imbibition.

The following exercises are intended to give you some understanding of the processes of diffusion, osmosis, and imbibition, and to engender your skills in teamwork between you and your lab partners.

Students will work in teams of four

Exercise A. Diffusion

A1. Heat: the motive force of diffusion

One member of each team to do set this up

Molecules diffuse in a fluid because the molecules of a liquid are in motion. While large objects in water are not visibly effected by this motion, small objects are. The random movement of very small objects due to molecular collisions in a fluid is called **Brownian motion**.

Procedure: Prepare a wet mount of homogenized milk. View the preparation at 400x. Note the tiny lipid droplets suspended in the aqueous medium. The movement you see is Brownian motion.

A2. Effect of molecular weight and temperature on the rate of diffusion

Two members of each team will do this activity.

Procedure:

- a. Obtain two small test tubes that are half filled with 5% gelatin .
- b. To one test tube, add 10 drops of 0.02 molar potassium dichromate ($K_2Cr_2O_7$), and to the other add 10 drops of 0.02 molar Janus green B ($C_{30}H_{31}ClN_6$).
- c. Cork the test tubes. Either place both in the tumbler labelled 'room temperature', or into the tumbler labelled '5 degrees C', as dictated by the instructions at your work station.



- d. Record the temperature of your room in degrees C using the thermometer on the side bench. Record the temperature in the table below.
- e. At the next class meeting, measure, in millimeters, the distance each solute has diffused into the gelatin. Determine how much time has lapsed since you started the experiment, and calculate a rate of movement through the gel for each condition in mm per hour. Record these rates below.

	Potassium dichromate	Janus Green
Room Temperature ____		
Refrigerator - 5 degrees C		

- f. Graph the relationship between temperature and distance (discussion activity).
- g. Graph the relationship between molecular mass and distance (discussion activity).

Explain the differences in the rates of diffusion among the four tubes.

Exercise B. Differentially Permeable Membranes.

Each person should view this demonstration

Dialysis tubing is differentially permeable. This property will be demonstrated by the following activity.

Procedure: Each lab section will do one setup. On the side bench is a test tube filled with a starch suspension sealed by a piece of dialysis tubing. At the beginning of the period your TA will invert the test tube into a dilute solution of I₂KI. Observe during the lab and also during the next lab period.

Is dialysis tubing permeable to iodine?

Is it permeable to starch?

What do you suppose causes dialysis tubing to be differential permeability (What is it that allows some things to move across but not others?)?

Exercise C. Osmosis.

C1. Osmotic pressure: Demonstration of an Osmometer

Each person to view this demonstration throughout the period

If the water concentration differs on either side of a differentially permeable membrane, the net movement of water will tend to go from the side with the higher water concentration to the one with the lower. This movement, however, can be stopped by positive pressure. The pressure required to stop the movement for a given system is that system's **osmotic pressure**. If that pressure is exceeded, water can be forced through the membrane against a concentration gradient; that is, from the side of lower concentration to the side with the higher. This method is used today to desalinate water and is also used in sophisticated home water purification systems.

The osmometer provides us with a way to measure osmotic pressure. Water will move into the compartment, or bag, and the solution will rise in the tube until equilibrium is reached. At this point, the solution in the tube exerts enough positive hydrostatic pressure, or "osmotic pressure," on the contents of the bag to equal, or offset, the negative osmotic potential of the sucrose solution.

In the osmometer on display, a dialysis bag containing a 40% sucrose solution to which a red dye has been added has been suspended in a beaker of distilled water. The bag is differentially permeable, allowing the passage of water but not the passage of sucrose.



- As you observe this demonstration, record the time and height of the column using the data sheet by osmometer.

Why does water move into the bag? _____

During osmosis, is water moving in only one direction (that is, only into the bag)?

Explain. _____

If the tube was 50 ft tall, what would eventually stop the net movement of water? (Be specific. Your understanding of this concept will be evaluated on the next quiz)

C2. Rates of osmosis

Two members of each team of four to do this activity

In this experiment, you will investigate the relationship between the rate of osmosis, the difference in solute concentrations, and temperature. You will place several dialysis bags containing solutions of different sucrose concentrations into beakers containing tap water at different temperatures. The rate and direction of water movement can be determined by measuring the change in the mass of the bags.

Procedure:

- Obtain three sections of dialysis tubing and place them in a beaker of distilled water.
- Fold over one end of each tube, tie tightly with string and return each tube to the distilled water.
- Open the untied end of one of one tube and add 7ml of 20% sucrose solution (49 M H₂O). Fold the end over, forcing out excess air, and secure it tightly with **blue** string.



- d.** Follow the same procedure with two other tubes, using a 50% sucrose solution (38.6 M H₂O) in one and tap water (55.6 M H₂O) in the other. Use **white** string for the water and **tan** string with a black mark for the 50% sucrose.
- e.** Weigh each tube. Record the mass on your data sheet.
- f.** Initiate the experiment by placing all three dialysis tubes into one 600 ml beaker of water. **In each lab, three groups to use water warmed to 35 degrees C (keep beaker in ice chest of warm water), and two groups to use ice water.**
- g.** After 1 hour and 15 minutes examine the tubes and record the results regarding their mass in the table below.

Treatment (circle one)	Cold		Hot	
Concentration at start (Sucrose)	Mass at start	Mass at end	Rate of change (g/minute)	Concentration at end
50% (38.6 M H ₂ O)				
20% (49 M H ₂ O)				
Tap Water (55.6 M H ₂ O)				

- h.** Determine the rate of change of the mass for each condition as follows:

$$\frac{\text{Mass (at the end)} - \text{Mass (at start)}}{75 \text{ minutes}} = \text{grams / minute}$$

Record these values:

Rate of change - grams (Tap water) _____

Rate of change - grams (20%) _____

Rate of change - grams (50%) _____

- i. Pool your data with your classmates, and calculate average rates of change in the mass of tubes under different treatments and fill in the tables below (Discussion activity).

Concentration at start (Sucrose)	Average change in mass (Hot)	Average change in mass (Cold)	Average change in mass (both)
50% (38.6 M H ₂ O)			
20% (49 M H ₂ O)			
Tap Water (55.6 M H ₂ O)			

- j. Graph the relationship between molar concentration and average rate of change in mass for the ‘hot’ treatments (Discussion activity).
- k. Graph the relationship between temperature and average rate of change for the 50% (38.6 M H₂O) treatments (Discussion activity).

What was the purpose of the tube filled with tap water?

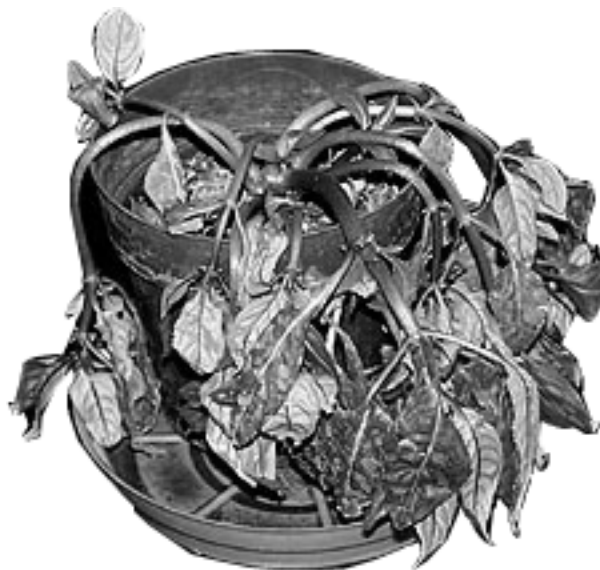
C3. Plasmolysis

Typically, in healthy plants, the solution around the plant’s cells is hypotonic with water tending to move into the cell. The pressure resulting from the protoplast pushing against the cell wall due to osmosis is called **turgor pressure**. This pressure is important to herbaceous plants. Without turgor pressure plants wilt.

C3a. The role of turgor pressure in providing support to herbaceous plants.

Each person to view this demonstration

Your TA will water the wilted plant with distilled water. Observe its recovery during the period.



What would happen if she had watered the plant with a hypertonic solution?

C3b. Observation of plasmolysis and recovery of plant cells

Two members of each team of four to do this

Procedure

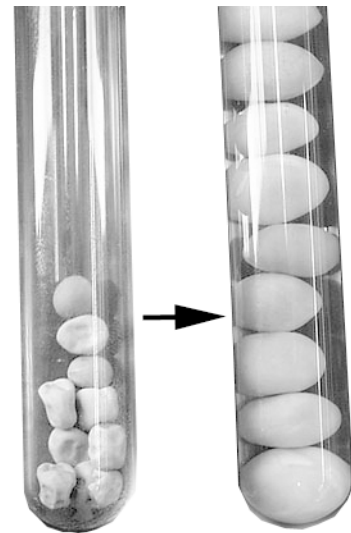
- a. Prepare a microslide of a leaf of *Elodea* in distilled water. Observe the condition of the cells, and sketch a cell near the edge of a leaf.
- b. Place a piece of tissue paper on one side of the coverslip and blot up the water while adding 40% sucrose solution on the other side.
- c. Observe the leaf cell sketched earlier as it contracts and pulls away from its cell wall. Sketch the cell in this condition (on the next page)
- e. Place a tissue on one side of the coverslip to blot up the 40% sucrose solution while adding distilled water on the other side. Observe the cell as it recovers. Sketch the same cell again.

Cell - original condition	Plasmolyzed cell	Cell after recovery

Exercise D. Imbibition and the Diffusion of Water

Each person should view this demonstration

At the time indicated in this demonstration, 10 dry pea seeds were placed in each of six test tubes. Tube 1 contains only dry seeds and will serve as a control. The other tubes were set up as follows: tube 2 was filled with petroleum ether, tube 3 with 50% sucrose solution, and tube 4 with distilled water. A day later, a similar series of test tubes was set up. Compare the differences between the two sets of tubes and the differences between the tubes within each set.



Why do the seeds in various solutions swell at different rates?

Why do the seeds in petroleum ether fail to swell?

What is imbibition?

What is osmosis?

What is the difference between diffusion and osmosis?

- between osmosis and imbibition?

- between imbibition and diffusion?

Master Data Sheet

Section # _____

Rate of Diffusion

Solute	Average Rate of Movement all conditions (mm/hour)	Average Rate of Movement cold (mm/hour)	Average Rate of Movement room temp (mm/hour)
Janus Green			
Potassium Dichromate			

Rate of Osmosis

Concentration at start	Average change in mass (Hot) g/minute	Average change in mass (Cold) g/minute	Average change in mass (both) g/minute
50%			
20%			
Distilled Water			

