

Biology 3A Laboratory: Membrane Transport

Objectives

- To observe Brownian motion and understand its importance in diffusion
- To study the movement of diffusion of different materials in various media
- To understand the effect of osmotic concentration on plant and animal cells
- To study diffusion through membranes
- To relate the movement of molecules (materials) to living systems
- To investigate factors affecting cell size

Introduction

All living things require essential materials to ensure their survival. Many chemical, physical and biological processes are utilized to obtain these materials for cells. These processes can be passive, occurring as a result of basic physical laws and require no outside energy; or they can be active, requiring energy expenditure (active transport). Waste materials can also be removed from cells via these same processes.

Since all molecules possess kinetic energy (energy of motion due to their electron movement), they have the ability to move randomly at very high speeds. Thus, diffusion is the passive movement of molecules from an area of higher concentration to areas of lower concentration. In a closed environment, molecules will disperse until they reach a state a dynamic equilibrium. As the molecules approach equilibrium, the net or overall rate of diffusion begins to slow and occurs equally in all directions. In an open environment, there are no "walls" to confine the molecules; therefore the molecules will always appear to move away from the immediate source. For instance, odors in a confined room persist much longer, whereas odors outside will dissipate more quickly. Temperature directly affects the rate of diffusion, whereas molecular weight indirectly affects the rate. From Graham's Law of Effusion, we know that increased temperatures will increase the rate of diffusion and the larger molecules will move more slowly than smaller molecules at the same temperature. In this lab, you will examine some of the basic physical principles accounting for the movement of molecules.

PART A: BROWNIAN MOTION

Brownian motion is the random movement of molecules due to kinetic energy. At normal environmental temperatures, all matter is in a constant state of motion. You may not see the movement of molecules in a gas or a liquid; however, they are under constant bombardment from other molecules, constantly colliding with each other. Even in a solid, molecules are vibrating. Certain molecules within our cells are kept in a suspended state due to the constant bombardment with the protoplasm (mainly by water, which they are dissolved in). In 1827, Robert Brown observed evidence of this molecular motion when viewing the debris along the outer edge of crushed leaves under a microscope.

A1 Procedure: Your instructor will set this up as a demonstration for the entire class

1. Place one drop each of: milk, India ink and water on a clean slide and cover with a cover slip. NOTE: The milk was boiled prior to the laboratory period.
2. Focus on the fat droplets in under low power. Then switch to high power and focus on the fat droplets.
3. Move the light source closer to the slide and note any difference in molecular movement after a few minutes.
4. Record your observations and answers on the lab worksheet.

PART B: DIFFUSION RATE of KMnO_4

In this experiment you will examine the diffusion rate of KMnO_4 into a liquid by using the spectrophotometer to quantify the samples collected. Remember, your absorbance reading will be directly proportional to the concentration of the sample.

B1 Procedure:

1. Turn on the spectrophotometer and allow it to warm up for 15 minutes.
2. Set the wavelength to 540 nm (maximum absorbance) and zero with a cuvette $\frac{3}{4}$ filled with DI water. Do not forget to check the spectrophotometer between readings due to "drift" in the accuracy of each instrument.
3. Obtain an 8" length of dialysis tubing that has been pre-soaked in DI water for a few minutes.
4. Tie off one end of the dialysis tubing by folding one end several times and placing one of the green or orange dialysis clips on the folded tubing.
5. Fill the tubing $\frac{3}{4}$ full of concentrated KMnO_4 . Carefully rinse the outside of the tubing and check to see that there are no leaks.
6. Place the tubing in a beaker containing 500 ml of DI water. Secure the tube to the beaker with a rubber band as shown by your instructor (Figure 1).
7. Turn the stirrer on at a low speed. **Do not** alter the speed of the stirrer during the experiment.
8. Fill a test tube $\frac{3}{4}$ full with a sample of the beaker water at 5-minute intervals for the first 15 minutes and then every 10 minutes for a total of 90 minutes.
9. Determine the absorbance of each sample at 540 nm.
10. Carefully return each sample to the beaker immediately after determining the absorbance.
11. Construct an appropriate graph in Excel using the data collected.

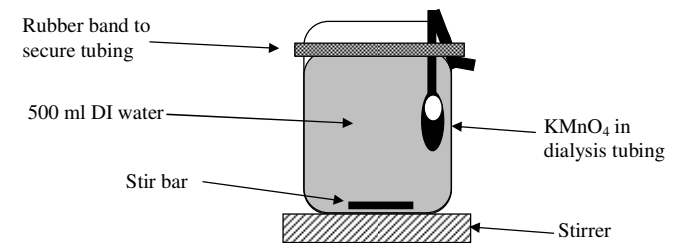


Figure 1. The apparatus for the diffusion of KMnO_4 experiment.

Part C: DIFFUSION OF LIQUID INTO A SEMI-SOLID

In this experiment, you will examine the effects of temperature and molecular weight of liquid molecules diffusing through a semi-solid, agar. The agar used is a non-nutrient colloid (gel). The molecular weight of methylene blue is 320. The molecular weight of potassium permanganate (KMnO_4) is 158. The molecular weight for methyl red is 269. The molecular weight of methyl violet is 394.

C1 Procedure:

1. Obtain a petri dish with the non-nutrient agar.
2. Using a #6 core borer, punch two holes in the agar as indicated by Figure 2.
3. Carefully remove the plug with a pair of forceps or dissecting needles. Make sure the edges are smooth and clean.
4. Measure the diameter of the holes in **millimeters (mm)**. All holes should be the same diameter.
5. Carefully fill a hole with the assigned dye. Do not over fill the wells.
6. Place a cover over the petri dish and place in the appropriate temperature environment as indicated by your instructor: **ice bath, room temperature (desk) or incubator (40C)**.
7. Examine your petri dish every 15 minutes and replenish the chemicals as needed. Measure the outside diameter of each ring in **millimeters (mm)**.
8. Allow the experiment to proceed for 90 minutes.
9. Record you data on your answer sheet and on the computer for the others.
10. Place the petri dish on the back counter in the hazardous waste container for proper disposal.
11. Construct an appropriate graph using Excel for the diffusion of all the dye molecules (using data from the whole class downloaded from the webpage).

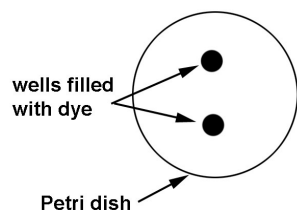


Figure 2. Petri dish with wells bored and filled with dye

PART D: OSMOSIS

Osmosis is the simple (passive) diffusion of water across a semi-permeable membrane. Water flows from an area of high water concentration (hypo-osmotic solution – low solute concentration) to areas of lower water concentration (hyperosmotic solution – high solute concentration). As you may recall from lecture, water has a slight charge to it, therefore it does not pass through phospholipid bilayer. Instead, water diffuses through special carrier proteins called **aquaporins**. Remember, water always flows from a hypo-osmotic to a hyperosmotic region.

D1 Procedure:

The instructor will set up this osmosis experiment and assign a group to collect the for the class data every 15 minutes for 120 minutes.

1. Tightly tie off one end of the dialysis tubing.
2. Open and slide other end of dialysis tubing over the burette and tie off tightly.
3. Fill one dialysis bag with the 25% colored sucrose solution and the other with the 75%.
4. Place both dialysis bags into separate beakers of DI water.
5. Note the level that each solution begins in each osmometer.
6. Record the levels for each: every 15 minutes for 120 minutes.

PART E: PLASMOLYSIS

Plant cells are surrounded by rigid cell walls, and under normal conditions, the cell membrane is pressed tightly against these cell walls. When plant cells are placed into an **iso-osmotic** (equal solute concentration) solution, the amount of water that enters the cells and leaves the cells is equal. When plant

cells are placed into a **hypo-osmotic** (lower solution concentration) solution, water will continue to diffuse in both directions across the membrane; however more of the water from outside the cells will enter the cells, causing them to become turgid (rigid). When plant cells are placed into a **hyperosmotic** (greater solute concentration) solution, plant cells will lose water at a higher rate than it gains water, causing the cell membranes to separate from the cell walls (**plasmolysis**), resulting in a flaccid plant.

NOTE:

- 0.9% NaCl concentration will represent an iso-osmotic solution (same as the cell)
- 0% NaCl concentration will represent a hypo-osmotic solution (less than the cell)
- 10% NaCl concentration will represent a hyperosmotic solution (greater than the cell)

E1 Procedure: Your instructor may set this up as a demonstration for the entire class

Make a wet mount of the Elodea leaf.

1. Notice and draw the arrangement of a plant cell.
2. Add a few drops of 10% NaCl solution to one end of the cover slip.
3. Place a paper towel lightly at the opposite side of the cover slip to draw the 10% NaCl solution towards it. You may have to add more NaCl solution. Wait 5 – 10 minutes.
4. Be patient. Notice and draw the arrangement of a plant cell (with detail) at 400X.
5. Add several drops of DI water to one end of the slide with the plasmolyzed plant cells.
6. Place a paper towel lightly at the opposite side of the cover slip to draw the DI water solution towards it. You may have to add more DI water. Wait 5 – 10 minutes.
7. Be patient. Notice and draw the arrangement of a plant cell.
8. Be sure to **clean** the slides and microscope thoroughly. **Reminder:** use only lens paper to clean the microscope lenses.

PART F: ANIMAL CELL PERMEABILITY

In this exercise, you examined how osmotic concentration affects animal cells. The solutions that you will use will cover a range of hypo-osmotic to hyperosmotic solutions relative to the red blood cell. You will determine the time it takes for red blood cells to hemolyze under varying conditions.

1. Your instructor will set up this experiment, and you will observe the results.
2. Add 2.0 ml of the following solutions to a series of test tubes according to Table 1 to the 5 mini-tubes.
3. Determine the time it takes for red blood cells to hemolyze under varying solute concentrations.
4. **Do each tube one at a time. DO NOT do all 5 tubes simultaneously!** Add two drops of mammalian blood to the test tube you are testing. Immediately cover with parafilm, invert several times and start timing simultaneously.
5. Immediately after inverting, place the test tube in front of a piece of white paper with printing on it and stop timing when you can read through the tube to the print. Record the time. Your instructor may demonstrate this. When you can clearly see letters

Table 1. Molar concentrations of NaCl for each mini-tube.

Tube #	NaCl (M)
1	0.05
2	0.08
3	0.15
4	0.30
5	1.0

through the solution, hemolysis is complete. If a solution does not clear within 3 minutes, then hemolysis will not occur (for this set of solutions)

- Microscope observations: Go online and “Goggle” search wet mount slides of the mammalian blood showing red blood cells in iso-osmotic, hypo-osmotic and hyperosmotic solutions.
- Describe (or sketch) their appearance and provide an explanation of the shape of the red blood cell.

What is going on in the tube when the blood mixture clears? This is hemolysis (cells bursting open).

What is going on in the tube when the blood mixture is cloudy? In this case the red blood cell is intact. But again, at some concentrations, the solution on the outside of the red blood cell is hypertonic.

PART G: DIALYSIS THROUGH NON-LIVING MEMBRANES

Dialysis is the diffusion of a dissolved substance through a semi-permeable membrane. Like simple diffusion, the solute will move from an area of higher concentration to an area of lower concentration through a semi-permeable membrane. In this experiment, you will examine the differential permeability that exists in cell membranes by using an artificial cell membrane (dialysis tubing). The dialysis tubing will contain various solutions that will be immersed in a beaker containing DI water and iodine.

Procedure:

- Prepare a 20 cm section of dialysis bag by soaking it in DI water for 5 – 10 minutes (if not pre-soaked).
- Tie off one end of the dialysis bag (twist the bag several times before tying off – overhand knot works well).
- Fill the dialysis bag with 5 ml of glucose, 5 ml of 2% starch & 2% protein solutions.
- Press out most (not all) of the air and tie off the other end like before (the bag should be flaccid).
- Carefully rinse the dialysis bag with DI water, blot dry and weigh to the nearest 0.1 g
- Record the weight of the bag on your worksheet.
- Fill a 600 ml beaker ½ full with DI water and 50 ml of 10% NaCl solution.
- Add the bag to the beaker. Every 15 – 20 minutes, carefully stir the bag in the beaker.
- Let the dialysis occur for at least 1 hour or when your instructor tells you to remove it.
- While the dialysis is occurring, set up 4 test tube with labels: IB1, IB2, IB3 & IB4 (IB = Inside Bag)
- Set up another 4 test tubes with labels: OB1, OB2, OB3 & OB4 (OB = Outside Bag).
- After the dialysis has occurred for at least an hour, remove the bag, blot dry, weigh it and record its mass on your worksheet.
- Using chemical indicators, you will determine which substance passed through the dialysis membrane. Carefully pour the contents of the bag equally into the four test tubes labeled IB1 – IB4 (INSIDE BAG).
- Pour the contents of the beaker in the four test tubes labeled OB1 – OB4 (OUTSIDE BAG), 5 ml each.
- Table 2 shows both positive and negative indicator test results.
 - Testing for NaCl: IB1 & OB1: Add 2 – 3 drops of the AgNO₃ solution and mix.
 - Testing for Glucose: IB2 & OB2: Add 1 ml of Benedict’s to each, mix and heat
 - Testing for protein: IB3 & OB3: Add 1 ml of Biuret to each test tube and mix.
 - Testing for starch: IB4 & OB4: Add 2 – 3 drops of Lugol’s Iodine solution

Table 2. Positive and negative results for various indicator solutions.

Solute	Indicator Solution	(+) or (-) Result	Color change
NaCl	AgNO ₃ solution	+	White precipitate
		-	No precipitate
Glucose	Benedict’s solution	+	Green/yellow/orange/red
		-	Blue
Protein	Biuret	+	Purple/pink (a.a.)
		-	No color change
Starch	Lugol’s iodine	+	Blue-black color
		-	Amber color (brownish)

PART H: Why don’t cells grow larger?

In this section you will examine the effects of placing four different sized cells into a “feeding” solution to gain insight as to why cells do not grow larger by analyzing surface area to volume ratios as it relates to cell division and nutrient uptake by simple diffusion. Your cell models were made from a phenol red agar poured into three different sized test tubes (15 mm, 20 mm and 25 mm). The tubes were capped tightly, inverted and allowed to solidify.

H1 Procedure:

- To remove the solidified cylinders, heat the bottom of each tube in boiling water. This will cause the air to expand and push the agar out of the tube far enough for extraction. **Carefully** remove the agar without tearing.
- Cut the two largest diameter cylinders to 3 cm, cell models #1 and #2. Cut the smallest diameter cylinder to 2 cm and 1 cm, cell models #3 and #4. Leave excess agar for another group.
- Record the cell identifying number, diameter and length in the “Before Feeding” section of Data Table 5.
- Place the cell models carefully into a finger bowl and cover with vinegar (simulated nutrient) until the largest cell is covered.
- Allow the cells to “feed” for about ten minutes.
- Measure the diameter and length of the remaining colored cylinder (red), representing the unfed portion of each cell. Place your measurements in the “After Feeding” section of Data Table 5.