

Lab 4: Diffusion and Membranes

BIOL-8

Name: _____ Date: _____

Learning Connections

Why This Matters for Human Biology:

Every cell in your body depends on diffusion and osmosis to survive. These processes explain:

- **Breathing:** How oxygen moves from your lungs into your blood, and CO₂ moves out
- **Digestion:** How nutrients are absorbed from your intestines into your bloodstream
- **Kidney Function:** How your kidneys filter waste and regulate water balance
- **IV Fluids:** Why medical IV solutions must be carefully formulated to match blood concentration
- **Dehydration:** Why drinking seawater makes you MORE dehydrated (osmosis pulls water OUT of cells)

In this lab, you'll see these same principles in action using dyes, agar, and dialysis tubing—models that help us understand what happens at the cellular level.

Objectives

By the end of this lab, you will be able to:

- **Define diffusion** and explain how molecular size affects diffusion rate
- **Investigate diffusion through a semi-solid medium** using an agar plate
- **Demonstrate temperature effects** on diffusion rate in liquids
- **Observe density-driven flow** patterns using temperature differences
- **Model osmosis** using dialysis tubing and solutions of varying sucrose concentrations
- **Demonstrate selective permeability** using the starch-iodine test
- **Apply diffusion concepts** to biological systems and human physiology

Introduction

Diffusion is the movement of molecules from an area of higher concentration to an area of lower concentration. This passive process requires no energy input and is fundamental to life—it's how oxygen enters cells, how carbon dioxide leaves, how nutrients are absorbed, and how wastes are removed.

Key Concepts:

- **Diffusion:** Net movement of molecules from high to low concentration
- **Osmosis:** Diffusion of water across a selectively permeable membrane
- **Selectively Permeable Membrane:** A membrane that allows some substances to pass while blocking others
- **Concentration Gradient:** The difference in concentration between two areas

Factors Affecting Diffusion Rate:

1. **Temperature** — Higher temperature = faster diffusion (molecules have more kinetic energy)
2. **Molecular Size** — Smaller molecules = faster diffusion
3. **Concentration Gradient** — Steeper gradient = faster diffusion
4. **Medium** — Diffusion is faster in gases than liquids, faster in liquids than solids

Part 1: Molecular Size and Diffusion Rate (Agar Plate)

Learning Goal: Compare how molecules of different sizes diffuse through a semi-solid medium.

Background

Smaller molecules move faster and diffuse more quickly than larger molecules. In this experiment, you'll observe how two different dyes with different molecular weights diffuse outward from a central well in an agar plate.

Dyes Used in This Experiment:

| Dye | Approximate Molecular Weight (g/mol) | Color |
|---|--------------------------------------|--------|
| Potassium permanganate (KMnO ₄) | 158 | Purple |
| Methylene blue | 320 | Blue |

Note: Methylene blue is approximately **twice** the molecular weight of potassium permanganate!

Materials Checklist

Before beginning Part 1, confirm you have the following materials:

- ☐ Agar plate with central well
- ☐ Potassium permanganate (KMnO₄) dye solution
- ☐ Methylene blue dye solution
- ☐ Ruler (mm scale)
- ☐ Timer or stopwatch
- ☐ Lab notebook for recording observations

Procedure

1. Obtain an agar plate with a central well
2. Carefully add a small drop of **potassium permanganate** (purple) to the well
3. Carefully add a small drop of **methylene blue** (blue) to the well
4. Record the **starting time**
5. Measure the **radius of diffusion** from the edge of the well to the leading edge of each dye
6. Record measurements at **10, 20, 30, 40, 50, and 60 minutes**

Hypothesis

Before you begin, predict which dye will diffuse faster. Explain your reasoning based on molecular weight:

Data Collection

Diffusion Radius (mm from well center)

| # | Dye | 10 min | 20 min | 30 min | 40 min | 50 min | 60 min |
|---|-----|--------|--------|--------|--------|--------|--------|
| 1 | | | | | | | |
| 2 | | | | | | | |

Analysis

1. Which dye diffused farther in 60 minutes? How does this relate to its molecular weight?

2. Calculate the average diffusion rate for each dye (final radius ÷ 60 minutes):

- Potassium permanganate (KMnO₄): mm/min
- Methylene blue: mm/min

3. The molecular weight of methylene blue is roughly double that of potassium permanganate. Did the diffusion rate reflect this size difference? Explain:

4. How does this relate to biological systems? Why can small oxygen molecules (O₂, MW = 32) diffuse across membranes easily while proteins (MW = thousands) cannot?

Part 2: Temperature and Diffusion in Water

Learning Goal: Investigate how temperature affects the rate of diffusion and observe density-driven flow patterns.

Background

At higher temperatures, molecules have more kinetic energy and move faster, resulting in faster diffusion. Additionally, temperature affects water density—hot water is less dense and rises, while cold water is denser and sinks. This experiment demonstrates both concepts.

Materials Checklist

Before beginning Part 2, confirm you have the following materials:

- ☐ Heating plate
- ☐ 2 beakers (250 mL each)
- ☐ Hot water (from hot plate, ~50-60°C)
- ☐ Ice water (from ice machine in chemical room corner)
- ☐ Red dye
- ☐ Blue dye
- ☐ Plastic frame with removable vertical divider
- ☐ White background paper or mat
- ☐ Stirring rod
- ☐ Thermometer (optional)

Part 2A: Temperature Effect on Diffusion

Procedure:

1. Prepare two beakers: one with hot water, one with ice water
2. Place both beakers on a white background for better visibility
3. Add **one drop of red dye** to the hot water — **do NOT stir**
4. Add **one drop of blue dye** to the cold water — **do NOT stir**
5. Observe how the dye spreads in each beaker

Observations — Part 2A

1. Which beaker showed faster diffusion of the dye? Describe what you observed:

2. Explain why temperature affects diffusion rate in terms of molecular kinetic energy:

Part 2B: Density Stratification Demonstration

Procedure:

1. After observing diffusion, **stir both beakers** to fully mix the dye
2. Set up the plastic frame with the vertical **divider firmly in place** in the middle
3. Carefully **pour the red-dyed hot water** on one side of the divider
4. Carefully **pour the blue-dyed cold water** on the other side
5. **Remove the divider slowly** and observe what happens to the colored water

Observations — Part 2B (Divider Removal)

1. When you removed the divider, what happened to the blue (cold) water and the red (hot) water? Describe the pattern you observed:

2. After the water stabilized, which color was on top and which was on bottom? Explain why this occurred:

Top layer color: — Temperature:

Bottom layer color: — Temperature:

Explanation:

Part 2C: Mixing One Side

Procedure:

1. While the red and blue layers are still visually stratified, carefully **replace the divider** in the middle
2. Using a stirring rod, **stir only ONE side** of the divider
3. You should now have one side stratified (red on top, blue on bottom) and one side mixed (purple throughout)
4. **Remove the divider again** and observe

Observations — Part 2C

1. After stirring one side, describe the colors on each side of the divider:

Stratified side:

Mixed side:

2. When you removed the divider, where did the purple (mixed) water flow?

Describe the flow pattern:

3. Explain why the purple water flowed to that location in terms of temperature and density:

Part 3: Osmosis and Dialysis Tubing

Learning Goal: *Demonstrate osmosis using dialysis tubing and demonstrate selective permeability.*

Background

Dialysis tubing is a selectively permeable membrane that allows water and small molecules (like iodine) to pass through but blocks larger molecules (like sucrose and starch). This makes it an excellent model for studying osmosis and selective permeability.

Key Terms — Understanding Direction:

When comparing two solutions separated by a membrane, we describe one solution *relative to* the other:

- **Hypertonic:** A solution with **higher solute concentration** than the solution it's compared to. Water moves **out of** the less concentrated side **into** the hypertonic side.
- **Hypotonic:** A solution with **lower solute concentration** than the solution it's compared to. Water moves **into** the less concentrated (hypotonic) side.
- **Isotonic:** Solutions have **equal solute concentrations**. No net water movement occurs.

Example: *If a dialysis bag contains 20% sucrose and is placed in a 1% sucrose bath:*

- The **bag** is hypertonic to the bath (higher solute inside)
- The **bath** is hypotonic to the bag (lower solute outside)
- Water moves **into the bag** (from low solute → high solute)

Materials Checklist

Before beginning Part 3, confirm you have the following materials:

- [] 6 pieces of dialysis tubing
- [] String and plastic clips for sealing tubing
- [] Large bowl for the bath
- [] 1% sucrose solution
- [] 10% sucrose solution
- [] 20% sucrose solution

- [] Starch solution
- [] Iodine solution
- [] Paper towels
- [] Timer

Preparing Dialysis Tubing

Critical Instructions:

1. Each group will prepare **6 dialysis tubes** total
2. **Soak each piece of tubing** in water before use (makes it easier to open and seal)
3. **Seal one end** of each tube by:
 4. Carefully folding the end over
 5. Tying the folded portion **securely with string**
6. After filling, seal the top with a **plastic clip** (for hanging)

 **CRITICAL:** Your tubing must be **tightly sealed** to prevent leaks! Test for leaks before placing in the bath.

Part 3A: Sucrose Concentration and Osmosis

Setup:

1. Fill the main bowl with **1% sucrose solution** (this is the "bath")
2. Prepare **3 dialysis bags** containing:
 3. Bag A: **1% sucrose solution** (isotonic — same concentration as bath)
 4. Bag B: **10% sucrose solution** (bag is hypertonic to bath — water will move IN)
 5. Bag C: **20% sucrose solution** (bag is hypertonic to bath — water will move IN)
6. Seal all bags securely and **blot dry** with paper towel
7. **Weigh each bag** and record initial mass
8. Place all bags in the 1% sucrose bath
9. Wait **30 minutes** (or as directed)
10. Remove bags, blot dry, and record final mass

Hypothesis — Part 3A

Predict what will happen to each bag. Will it gain mass, lose mass, or stay the same? Explain your reasoning:

| Bag | Contents | Prediction | Reasoning |
|-----|-------------|----------------------|----------------------|
| A | 1% sucrose | <input type="text"/> | <input type="text"/> |
| B | 10% sucrose | <input type="text"/> | <input type="text"/> |
| C | 20% sucrose | <input type="text"/> | <input type="text"/> |

Data Collection — Part 3A

Dialysis Tubing Osmosis Data

| # | Bag | Contents (% Sucrose) | Initial Mass (g) | Final Mass (g) | Change in Mass (g) | % Change |
|---|-----|----------------------|------------------|----------------|--------------------|----------|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |

Calculate % Change:

$$\% \text{ Change} = [(\text{Final Mass} - \text{Initial Mass}) / \text{Initial Mass}] \times 100$$

Example: Initial = 10.0 g, Final = 12.5 g

$$\% \text{ Change} = [(12.5 - 10.0) / 10.0] \times 100 = +25\%$$

Analysis — Part 3A

1. Which bag(s) gained mass? Which lost mass? Which stayed about the same?

2. Explain your results in terms of osmosis. Which direction did water move for each bag and why?

3. For each bag, classify the INSIDE relative to the OUTSIDE (1% sucrose bath). Is the bag hypertonic to the bath, hypotonic to the bath, or isotonic?

| Bag | Inside Solution | Bag is _____ to the bath | Water moves _____ |
|------------|-----------------|--------------------------|-------------------|
| A (1%) | 1% sucrose | <div></div> | <div></div> |
| B (10%) | 10% sucrose | <div></div> | <div></div> |
| C (20%) | 20% sucrose | <div></div> | <div></div> |

Part 3B: Selective Permeability — Starch and Iodine Test

Background:

This experiment demonstrates that dialysis tubing is **selectively permeable**:

- **Iodine** molecules are small enough to pass through the membrane
- **Starch** molecules are too large to pass through

When iodine contacts starch, it turns **blue-black** (positive starch test).

Setup:

1. Prepare a dialysis bag containing **starch solution**
2. Seal the bag securely
3. Fill a beaker with water and add **iodine solution** (the bath will turn amber/brown)
4. Place the starch-filled dialysis bag in the iodine bath
5. Wait **15-20 minutes** and observe both the bag and the bath

Hypothesis — Part 3B

Predict what color changes you will observe. Explain your reasoning based on molecular size:

Inside the bag (starch solution):

Outside the bag (iodine solution):

Reasoning:

Observations — Part 3B

1. What color did the INSIDE of the dialysis bag become? What does this indicate?

2. What color was the SOLUTION OUTSIDE the bag? Did it change?

3. Based on your observations, which molecule passed through the dialysis membrane — starch or iodine? How do you know?

4. Explain why one molecule could pass through and the other could not:

Summary and Conclusions

1. List three factors that affect the rate of diffusion and explain how each factor affects it:

| Factor | How it Affects Diffusion Rate |
|--------|-------------------------------|
| | |
| | |
| | |

2. Compare and contrast diffusion and osmosis:

3. If a red blood cell were placed in distilled water (pure water, no solutes), would it swell, shrink, or stay the same? Explain using the terms hypertonic/hypotonic/isotonic:

4. Why is it important that IV (intravenous) fluids given to patients are isotonic with blood?

5. How do the processes studied today relate to human physiology? Give at least two specific examples:

6. What sources of error might have affected your results? How could you improve the experiments?

Quick Reference

Key Formulas

Formula

Rate = Distance / Time

% Change = [(Final - Initial) / Initial] × 100

Purpose

Calculate diffusion rate

Calculate percent change in mass

Tonicity Summary (Solution Relative to Cell)

| If the SURROUNDING solution is... | Solute Concentration | Water Movement | Cell Response |
|-----------------------------------|----------------------|-------------------|--------------------------|
| Hypertonic (to the cell) | Higher outside cell | Water OUT of cell | Cell shrinks (crenation) |
| Hypotonic (to the cell) | Lower outside cell | Water INTO cell | Cell swells (may lyse) |
| Isotonic (to the cell) | Equal inside/outside | No net movement | Cell stays same |

Molecular Weights Reference

| Molecule | Approximate MW (g/mol) | Can Pass Dialysis Membrane? |
|--------------------------|------------------------|-----------------------------|
| Water (H ₂ O) | 18 | Yes |
| Oxygen (O ₂) | 32 | Yes |

| Molecule | Approximate MW (g/mol) | Can Pass Dialysis Membrane? |
|--------------------------|-------------------------------|------------------------------------|
| Iodine (I ₂) | 254 | Yes |
| Potassium permanganate | 158 | Yes |
| Methylene blue | 320 | Yes (slowly) |
| Sucrose | 342 | No |
| Starch | ~1,000+ | No |
| Proteins | 10,000+ | No |

Connection to Module 04: Understanding diffusion and osmosis is essential for understanding how cells function. These passive transport processes explain how oxygen enters your blood, how nutrients are absorbed in your intestines, how your kidneys filter waste, and why drinking seawater dehydrates you. Membrane transport is fundamental to life!

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