Lab 4: Diffusion and Membranes

BIOL-8

Name: ______________________ **Date:** ______________________

Name: [ ] **Date:** [ ]

Objectives

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

- **Define diffusion** and explain what factors affect the rate of diffusion
- **Investigate how temperature affects** the rate of dye diffusion in liquids
- **Compare diffusion rates of molecules** with different molecular weights using agar plates
- **Demonstrate osmosis** using dialysis tubing and solutions of varying concentrations
- **Distinguish between hypertonic, hypotonic, and isotonic** solutions
- **Apply diffusion concepts** to biological systems and human physiology

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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
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

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Part 1: Temperature and Diffusion Rate

> ***Learning Goal:*** *Investigate how temperature affects the rate of dye diffusion in water.*

Background

At higher temperatures, molecules have more kinetic energy and move faster. This should result in faster diffusion. In this experiment, you will compare how quickly dye spreads in water at three different temperatures.

Materials

- 3 beakers (250 mL) with water at different temperatures:
- Cold water (ice water, ~5°C)
- Room temperature water (~20-25°C)
- Warm water (~40-45°C)
- Food coloring or dye
- Thermometer
- Timer/stopwatch
- Ruler

Procedure

1. Measure and record the **exact temperature** of each beaker
2. Place all three beakers on a white surface where you can observe them clearly
3. Simultaneously (or quickly in sequence) add **ONE drop** of dye to each beaker
4. **Do NOT stir** — let diffusion occur naturally
5. Observe and record the **diameter of the dye spread** at 1-minute intervals for 5 minutes

Hypothesis

Before you begin, predict which temperature will show the fastest diffusion. Explain your reasoning:

Data Collection

Temperature Measurements

#	Beaker	Target Temperature	Actual Temperature (°C)
1			
2			
3			

Dye Diffusion — Diameter of Spread (cm)

#	Beaker	1 min	2 min	3 min	4 min	5 min
1						
2						
3						

Graphing

Create a line graph plotting diameter of spread (y-axis) vs. time (x-axis) for all three temperatures. Use different colors or symbols for each temperature.

Graph Title: ___________

```
Diameter (cm)
    |
  8 ┤
    |
  7 ┤
    |
  6 ┤
    |
  5 ┤
    |
  4 ┤
    |
  3 ┤
    |
  2 ┤
    |
  1 ┤
    |
  0 ┼────┬────┬────┬────┬────┬────
         1    2    3    4    5    Time (min)
```

```
Key: Cold = ___   Room Temp = ___   Warm = ___
```

Analysis

1. Which temperature showed the fastest rate of diffusion? Was your hypothesis supported?

2. Calculate the rate of diffusion for each temperature by dividing the final diameter by 5 minutes:

- Cold water rate: ______ cm/min
- Room temp rate: ______ cm/min
- Warm water rate: ______ cm/min

3. Explain in terms of molecular motion why temperature affects diffusion rate:

4. Body temperature is 37°C. How does this relate to efficient diffusion of gases and nutrients in your body?

Part 2: 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 different dyes (with different molecular weights) diffuse outward from a central well in an agar plate.

Common Dyes and Approximate Molecular Weights:

Dye	Approximate Molecular Weight (g/mol)	Color
Potassium permanganate ($KMnO_4$)	158	Purple
Methylene blue	320	Blue
Janus green	511	Green
Congo red	697	Red

Materials

- Agar plate with central well (or multiple wells)
- 2-3 different dyes of varying molecular weights
- Ruler (mm)
- Timer

Procedure

1. Your instructor will provide an agar plate with wells cut into it
2. Add a small amount of each dye to separate wells (or add to same central well if designed that way)
3. Record the **starting time**
4. Measure the **radius of diffusion** from the edge of the well to the leading edge of the dye at regular intervals
5. Continue for 20-30 minutes or as directed

Hypothesis

Predict which dye will diffuse fastest. Explain based on molecular weight:

Data Collection

Record the dyes used:

Dyes Used

#	Dye Name	Molecular Weight (g/mol)	Color
1			
2			
3			

Diffusion Distance (mm from well edge)

#	Dye	5 min	10 min	15 min	20 min	25 min	30 min
1							
2							
3							

Draw Your Agar Plate

Sketch the diffusion pattern at the end of the experiment. Label each dye/color and show the relative diffusion distances.

```
       ___________________________
     /                             \
    /                               \
   |                                 |
   |                                 |
   |          ○ ← Well(s)            |
   |                                 |
   |                                 |
    \                               /
     \_____________________________ /

Labels: ___________________________
```

Analysis

1. Which dye diffused the farthest? What was its molecular weight?

2. Calculate the diffusion rate for each dye (total distance ÷ total time):

- Dye 1 (____________): ________ mm/min
- Dye 2 (____________): ________ mm/min

- Dye 3 ( ): mm/min

3. Is there a relationship between molecular weight and diffusion rate? Explain:

4. How does this relate to biological systems? For example, why can small oxygen molecules (O_2, MW = 32) diffuse across membranes easily while proteins (MW = thousands) cannot?

Part 3: Osmosis and Dialysis Tubing

> ***Learning Goal:*** *Demonstrate osmosis using dialysis tubing and solutions of varying sucrose concentrations.*

Background

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

Key Terms:

- **Hypertonic solution**: Higher solute concentration (water moves OUT of cell)
- **Hypotonic solution**: Lower solute concentration (water moves INTO cell)
- **Isotonic solution**: Equal solute concentration (no net water movement)

When dialysis bags containing sucrose solution are placed in water:

- Water moves **into** the bag (hypertonic inside relative to outside)
- The bag **gains mass**

When dialysis bags containing water are placed in sucrose solution:

- Water moves **out of** the bag (hypotonic inside relative to outside)
- The bag **loses mass**

Materials

- Dialysis tubing (pre-soaked)
- String or clips to close tubing ends
- Balance
- Beakers (4)
- Sucrose solutions: 0%, 10%, 20%, 30%
- Paper towels
- Timer

Procedure

1. **Prepare 4 dialysis bags:**
2. Bag A: Fill with 0% sucrose (distilled water)
3. Bag B: Fill with 10% sucrose solution
4. Bag C: Fill with 20% sucrose solution
5. Bag D: Fill with 30% sucrose solution
6. **Tie off both ends securely** — no leaks!
7. **Blot dry** with paper towel and record **initial mass**
8. **Place each bag in a beaker of distilled water (0% sucrose)**
9. **Wait 30 minutes** (or as directed)
10. **Remove bags, blot dry, and record final mass**

Hypothesis

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

Data Collection

Dialysis Tubing Osmosis Data

#	Bag	Contents (% Sucrose)	Initial Mass (g)	Final Mass (g)	Change in Mass (g)	% Change
1						
2						
3						
4						

Calculate % Change:

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

Example: Initial = 10.0 g, Final = 12.5 g
% Change = [(12.5 - 10.0) / 10.0] × 100 = 25%
```

Graphing

Create a bar graph showing % change in mass for each bag.

```
% Change in Mass
    |
+40 ┤
    |
+30 ┤
    |
+20 ┤
    |
```

```
+10 ┤
    |
  0 ┼──────────────────────────────────
    |
-10 ┤
    |
-20 ┤
         A         B         C         D
       (0%)      (10%)     (20%)     (30%)
                 Bag (% Sucrose Inside)
```

Analysis

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 and why?

3. For each bag, classify the INSIDE of the bag relative to the beaker solution (distilled water):

Bag	Hypertonic, Hypotonic, or Isotonic (inside relative to outside)?
A (0%)	
B (10%)	
C (20%)	
D (30%)	

4. If a red blood cell were placed in distilled water, would it swell, shrink, or stay the same? Explain using the terms hypertonic/hypotonic/isotonic:

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

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

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

Quick Reference

Key Formulas

Formula	Purpose
Rate = Distance / Time	Calculate diffusion rate
% Change = [(Final - Initial) / Initial] × 100	Calculate percent change in mass

Tonicity Summary

Solution Type	Solute Concentration	Water Movement	Cell Response
Hypertonic	Higher outside cell	Water OUT	Cell shrinks (crenation)
Hypotonic	Lower outside cell	Water IN	Cell swells (may lyse)
Isotonic	Equal inside/outside	No net movement	Cell stays same

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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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