

Diffusion across Biological Membranes

Prior to lab you should understand:

- Understand the process of diffusion, osmosis, plasmolysis and deplasmolysis and how these processes relate to one another.
- Understand the factors that affect the rate of diffusion of substances across a plasma membrane.

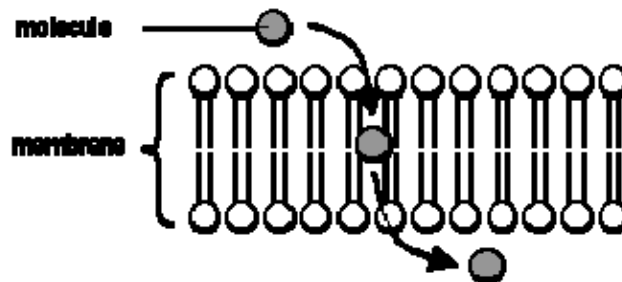
I. Objective:

- Determine which concentrations of sucrose are hypertonic, hypotonic and isotonic to elodea cells by assaying the solutions for their ability to induce plasmolysis.
- Investigate the properties of molecules which influence their rate of diffusion across biological membranes.

II. Background:

A. Diffusion

Biological membranes are described as **semipermeable**. This means that some molecules can pass through the membrane while other molecules cannot. Different types of molecules cross the membrane using various mechanisms. The simplest of these mechanisms is simple diffusion. During simple diffusion, molecules simply pass through the lipid bilayer component of the membrane.

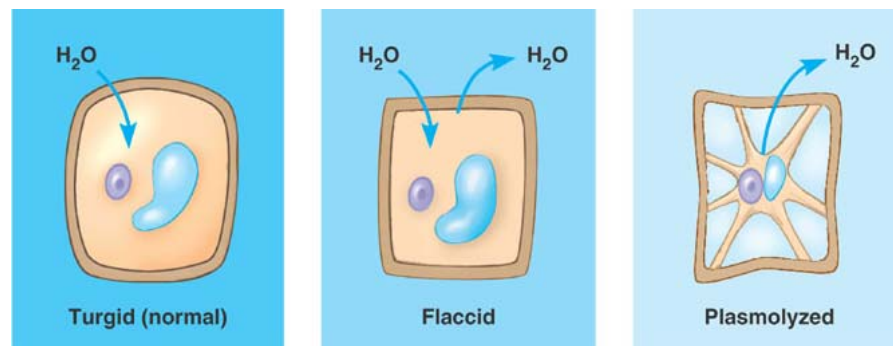


Molecules that move across the membrane using diffusion must either be very hydrophobic or they must be small and uncharged. For example, lipid-soluble molecules such as steroids, or very small molecules, such as H_2O , O_2 and CO_2 can diffuse rapidly across the membrane. Charged molecules or large polar molecules cannot move across the phospholipids bilayer by diffusion. Instead these molecules (e.g. amino acids, ions, disaccharides) must use a protein mediated mechanisms to cross the membrane such as facilitated diffusion or active transport. Since diffusion is a passive process, no energy is involved and substances can only move down their concentration gradient. Diffusion cannot be controlled by the cell, in the sense of being switched on or off. In this way it is different for facilitated diffusion and active transport which can be regulated by the cell.

B. Osmosis

Osmosis describes the diffusion of water across membranes. Because water is a small, uncharged molecule it can rapidly diffuse across biological membranes. Therefore, technically, osmosis is an example of simple diffusion. However, osmosis differs from other types of

diffusion, because it isn't the concentration of water that determines the direction of diffusion. Instead, it is the concentration of solutes dissolved in the water. The water moves from the side of the membrane with low solute concentration to the side of the membrane with high solute concentration. Consider the figure below from your textbook. It depicts a plant cell under different osmotic conditions. In the first panel, the cell is in a **hypotonic** solution (the concentration of solutes in the solution is lower than that in the cell). Under these conditions there is a net movement of water into the cells. Notice that the cell is depicted as swelling slightly because of the water moving into it. The plant cell's rigid cell wall prevents it from bursting. When an animal cell, which lacks a cell wall, is put into a hypotonic solution it would typically swell until it burst.

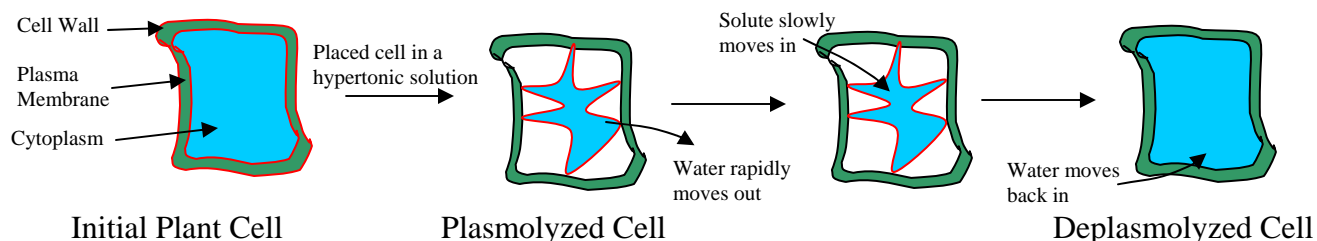


In the second panel, the cell has been placed into an **isotonic** solution (the solute concentration outside the cell is the same as inside the cell). Under these conditions there is no net movement of water inside or outside the cell. In the third panel, the cell has been placed in a **hypertonic** solution (the concentration of solutes in the solution is higher than inside the cell). Under these conditions the water moves outside of the cell. The cytoplasm decreases as the water exits the cell and the plasma membrane pulls away from the cell wall. This phenomenon is called **plasmolysis**.

C. Deplasmolysis

Not all diffusible molecules move across the membrane at the same rate. Some molecules move very rapidly across the membrane by diffusion, while others move very slowly. One assay to determine the rate of movement of a molecule across the membrane is to measure the rate of deplasmolysis. **Deplasmolysis** describes the behavior of cells that have been put into hypertonic solutions of molecules that can cross the membrane. Consider the diagram below. The cell in the first panel represents a typical cell before it is placed in a hypertonic solution. The second panel depicts the plasmolytic cell after it has been placed in a hypertonic solution and the water is moved out of the cell. If the solute in the hypertonic solution cannot cross the membrane, it will remain in this state. However, if the solute in the hypertonic solution can cross the membrane, it will diffuse from the area of high concentration (outside the cell) to an area of low concentration (inside the cell) as depicted in the third panel. Once the solute has diffused inside the cell, the plant cell will become hypotonic relative to the solution (from all the biomolecules dissolved in the cytoplasm). This causes the water to move back into the cell by osmosis, re-swelling the cytoplasm as depicted in the fourth panel. This re-swelling of the cytoplasm is called deplasmolysis.

Process of Plasmolysis



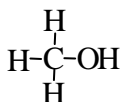
The initial step in this process, plasmolysis, is usually very rapid. This is because water can rapidly move across the membrane in response to a concentration gradient. The second phase, deplasmolysis, occurs at different rates depending on the chemical nature of the solvent in the solution. If the solvent can move across the membrane rapidly, then deplasmolysis can occur very quickly. If the solvent moves across the membrane more slowly, then deplasmolysis will occur more slowly. *Therefore the rate of deplasmolysis (i.e. the time from plasmolysis until the time the cell just begins reswelling) can be used as a measure of the rate of movement of solvents across the membrane.*

In this laboratory period, you will conduct two investigations.

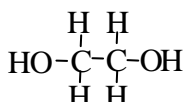
First, you will assay several concentrations of sucrose to determine which concentration induces plasmolysis in elodea leaves. From these data you will estimate which concentrations of sucrose are hypertonic, hypotonic and isotonic to elodea leaves.

Second, you will investigate two chemical characteristics which influence the rate of diffusion across the membrane, size of the molecule and hydrophobicity. To analyze the effect of size, you will compare the rate of deplasmolysis of three molecules with similar hydrophobicity but of different sizes.

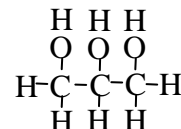
Methanol



Ethylene Glycol

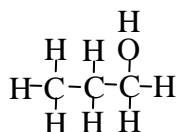


Glycerol

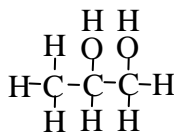


To analyze the effect of hydrophobicity, you will analyze three molecules with a similar size but with different hydrophobicities.

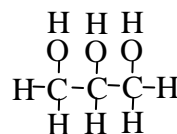
1-Propanol



Propylene glycol



Glycerol



III. Materials:

Each Station should have:

Light Microscope	Slides and Cover Slips
Elodea in water	Forceps
Kimwipes	Dropper of water
Droppers of 0.2M, 0.3M, 0.4M 0.5M and 0.6M Sucrose	
Droppers of alcohols in isotonic sucrose	
Methanol	Ethylene glycol
Propanol	Propylene glycol
Glycerol	
Waste dishes for used slides	

IV. Procedure:

A. Observing Plasmolysis

1. Prepare a wet mount of an Elodea leaf in Water
 - a. Remove a healthy leaf of elodea
 - b. Place leaf on a clean slide with upper surface facing up
 - c. Blot off excess water with kimwipe
 - d. Add two drops of water and a coverslip
2. Observe under high dry objective
3. Prepare a wet mount of an Elodea leaf in 0.6 M sucrose
 - a. Remove a healthy leaf of elodea
 - b. Place leaf on a clean slide with upper surface facing up
 - c. Blot off excess water with kimwipe
 - d. Add two drops of 0.6 M sucrose and a coverslip
4. Observe under high dry objective
5. Note the cellular changes associated with plasmolysis by comparing the structure of the cells mounted in water vs. 0.6M sucrose.
6. Prepare new wet mounts of elodea leaves in 0.2M, 0.3M, 0.4M sucrose.
7. Observe each slide periodically for every minute to monitor for signs of plasmolysis. Record when plasmolysis occurs in the data collection sheet. If after 5 minutes no plasmolysis occurs, record "none" on the data collection sheet.

C. Measuring the rate of deplasmolysis

1. Prepare a wet mount of an Elodea leaf in ethylene glycol using the procedure described above.
(Remember to blot excess water before adding ethylene glycol)
2. Record the time the mount is prepared
3. Observe under high dry objective
4. Observe and record the degree of plasmolysis
5. Watch for the first movement of water into the cell. Record this time.
(Note: if deplasmolysis doesn't occur in 10 minutes assume it will not occur. Record that deplasmolysis did not occur)
6. Repeat procedure for the other 4 alcohol solutions

V. Short Report

Name: _____

A. Plasmolysis Data Collection Sheet

Sucrose Concentration	Time after exposure plasmolysis first noted.
0.5 M	
0.4 M	
0.3 M	
0.2M	

B. Deplasmolysis Data Collection Sheet

Alcohol	Time wet mount is prepared	Extent of plasmolysis (none, slight, moderate, severe)	Time deplasmolysis begins	Elapsed time until plasmolysis begins (Min)
Ethylene Glycol				
Glycerol				
Methanol				
Propylene Glycol				
Propanol				

1. Based on the observations recorded in table A, which sucrose solution tested is most likely to be isotonic to elodea leaves? Explain how your data support your conclusion.

2. Based on your observations recorded in table B, what is the relationship between the hydrophobicity of a molecule and its rate of diffusion across the membrane? Explain how your data support your conclusion.

3. Based on your observations recorded in table B, what is the relationship between the size of a molecule and its rate of diffusion across the membrane? Explain how your data support your conclusion.