



THE BIOTECHNOLOGY
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Edvo-Kit #

S-74

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What is Osmosis?

Experiment Objective:

- Students will predict the net movement of molecules across a semipermeable membrane.
- Students will define what molecules move during diffusion and osmosis.
- Students will understand that osmosis and diffusion are important principles in animal and plant physiological systems.

See page 3 for storage instructions.

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Experiment Components

Contents

- Orange Dye, Low Molecular Weight Dye
- Blue/Green Dye, High Molecular Weight Dye
- 1 Roll of Dialysis Tubing (7.5 feet)
- String
- 5 Transfer pipets

Check (✓)

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Experiment #S-74 is designed for 5 groups.

Storage:
Store experiment at room temperature.

Requirements

- Table Salt
- Beakers or Jars (300-600 ml)
- Distilled or Deionized Water
- Spoon
- Apple Juice, Beet Juice, or Equivalent Juices

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Background Information

Molecules undergo constant motion, which enables them to move from one region to another. The motion of a molecule in solution is constantly affected by collisions with other molecules, which results in random motion. Diffusion, which is important to living systems, occurs in response to molecular motion and to a concentration gradient, that is, molecules moving from areas of high concentration to areas of low concentration until an equilibrium is reached. Examples include the oxygenation of blood in the lungs and the exchange of nutrients into cells and waste products out of cells.

During osmosis, the diffusion of water molecules down a concentration gradient occurs across a semi-permeable membrane. A semi-permeable membrane is a membrane which allows some materials to pass through while rejecting others. This is true of the plasma membrane surrounding all living cells. Dialysis tubing can be used to demonstrate the properties of a semi-permeable membrane in relation to osmosis. For example, if one fills a piece of dialysis tubing with a 5% salt solution (95% water) and places the tubing into a 100% water bath, water molecules will move from the water bath, a region with a higher concentration of water molecules, into the tubing which has a lower concentration of water molecules. The tubing, being semi-permeable, allows only the smaller water molecules to move through the pores. The membrane is impermeable to salt and therefore salt cannot move through the pores. The net result is a swelling in the bag size due to the influx of water molecules.

If the liquid in the dialysis tubing is 100% water and the bath outside the tubing is a 5% salt solution, then there will be a flux of water out of the tubing, which will result in shrinkage of the tubing. In the case where the concentration of water is equal on both sides of the dialysis membrane, that is, the liquid in the bath is the same concentration as that in the dialysis tubing, then no change in tubing will result. An equilibrium exists between the water molecules inside and outside the tubing (Figure 1).

Dialysis membranes are made of purified cellulose containing microscopic pores. The pore size is controlled during manufacturing. The pore size determines the membrane's permeability to molecules of different sizes. This assumes that these molecules have similar shapes. The dialysis tubing being used in this experiment will allow only low molecule weight molecules to pass through the membrane, while larger molecules will remain in the dialysis tubing.

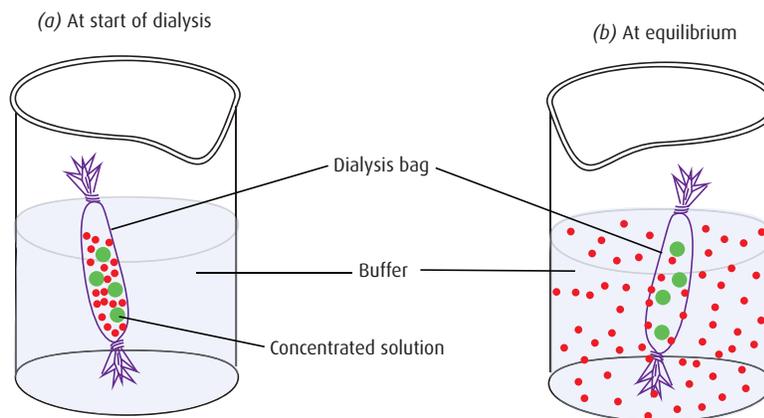


Figure 1: Movement of water and molecules during osmosis

Experiment Overview

EXPERIMENT OBJECTIVE:

- Students will predict the net movement of molecules across a semipermeable membrane.
- Students will define what molecules move during diffusion and osmosis.
- Students will understand that osmosis and diffusion are important principles in animal and plant physiological systems.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



WORKING HYPOTHESIS

If the movement of molecules is from a higher concentration to a lower concentration through a semi-permeable membrane, then qualitative results to demonstrate this principle can be observed in experimental procedures.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Activity One - Learning about Osmosis and Dialysis Membrane

WORKING HYPOTHESIS

If a semi-permeable membrane allows for molecules of a certain size to pass through, then one can determine relative sizes of colored molecules based on color changes in the surrounding solution.



EXPERIMENT PROCEDURE

1. Prepare two pieces of dialysis tubing as follows:

Use the string to close one end of the tubing by tying a tight knot at one end of moist dialysis tubing. Tie the string approximately one half inch from the end.

2. Prepare the dialysis bath by completely filling a clean beaker or jar with water.
3. Fill the first dialysis bag with blue/green dye:
 - Roll the untied end of the dialysis bag between your thumb and pointer finger to open the end of the bag.
 - Squeeze the transfer pipet bulb and fill it with dye up to a level just below the pipet bulb.
 - Gently insert tip of the transfer pipet deep into the bag.
 - Apply pressure on the bulb to transfer the contents of the pipet to the dialysis bag.
 - Carefully use the string to tie a tight knot at the open end of the bag. Place the sample in the beaker of water.
4. Rinse the transfer pipet in water and fill the second dialysis bag with orange dye. Follow the same procedures as in step 3.

You should now have two filled dialysis bags (one blue/green, and one orange) in a beaker of water.

5. After 15 minutes, observe if there is any color change in the dialysis bath water.
6. One at a time, remove each bag filled with dye and observe its contents. Is there any change in the color? Put the dialysis bags back in the beaker.
7. Periodically check the color of the bags and dialysis bath water and determine if any color change has occurred in either the bags or the bath water.
8. Which of the two dyes has a smaller molecular size? Why? Which has a larger molecular size.
9. Record your results.

Activity Two - Effect of Salt on Osmosis

WORKING HYPOTHESIS

If water molecules move from a higher concentration of water molecules to a lower concentration, then a dialysis bag containing a colored dye and placed into a salt solution, will shrink in size if the concentration of water molecules is higher in the bag than in the surrounding solution.



EXPERIMENT PROCEDURE

1. Prepare a fresh dialysis bath as described in Activity One.
2. Add 3 Tablespoons of salt to the dialysis bath. Stir until the salt is dissolved.
3. From Activity One, obtain the dialysis bag that still contains dye.
Hint: This is the high molecular weight dye, and it did not pass through the dialysis membrane.
4. Place the dialysis bag saved from Activity One into the dialysis bath that now contains salt.
5. After 15 minutes, observe if there is any change in the dialysis bag. Has the color changed? Does the volume of the bag appear the same or different?
6. Periodically check to see if any changes in color or volume has occurred.
7. Initially, there will be a flux of water out of the dialysis bag into the bath, which has a high salt concentration. This will result in shrinkage of the volume inside the bag. What happens to the dye when the volume shrinks?
8. Record your Results

Activity Three - Dialysis of Juice Pigments

WORKING HYPOTHESIS

If a semi-permeable membrane allows for molecules of a certain size to pass through, then one can determine relative sizes of juice pigments based on color changes in the surrounding solution.



EXPERIMENT PROCEDURE

1. Obtain a clear, non-particulate fruit or vegetable juice that contains a pigment, such as beet, cranberry, or apple juice. Each group can use the same juice, or can choose different juices.
2. Prepare a fresh dialysis bag and a fresh dialysis bath as in Activity One.
3. Prepare the dialysis bag with the juice your group has chosen and place it in the dialysis bath.
4. After 15 minutes, observe if there is any color change in the dialysis bath water or dialysis bag.
5. Periodically check the color of the bags and dialysis bath water and determine if any color change has occurred in either the bags or the bath water.
6. From the dialysis, it is possible to visually determine the relative size of some dye molecules. In this activity, the semi-permeable membrane will permit the passage of molecules which are small, but will not allow the passage of large ones.
7. What can you tell about the size of the juice pigment chosen by your group? Compare your group's result with the results from the other four groups in your class.
8. Record your Results

Study Questions

1. Differentiate between diffusion and osmosis.
2. What evidence from this experiment supports the idea that molecules are constantly in motion?
3. What is meant by a semi-permeable membrane?
4. What determines a membrane's permeability to molecules of different sizes? Give a concrete example from Activity #1.
5. In Activity #2, what happened to the overall size of a dialysis bag when placed into a salt dialysis bath? Explain.
6. Give examples from Activity #3 of different juice pigments that pass through and those that do not pass through a semi-permeable membrane.

Instructor's Guide

PRE-LAB PREPARATIONS

1. Cut the dialysis tubing into smaller pieces - each piece should be about 3 inches long. You should get at least 30 pieces from the tubing provided.
2. Cut the string into smaller pieces. Each piece should be about 3 inches long. You should get at least 60 pieces from the string provided.
3. Place the cut tubing in a clean beaker or jar filled with distilled water. Soak the tubing for at least one hour or overnight.
4. Each group requires approximately 3 ml of the dye solutions for Activity One. The solutions can be dispensed into individual tubes or placed at a classroom pipetting station for students to share.
5. Each group requires three tablespoons of salt for Activity Two. The salt can be dispensed into individual cups or placed at a classroom pipetting station for students to share.
6. Each group requires approximately 3 mL of a clear, non-particulate fruit or vegetable juice. Students can use the same juice or groups can choose different juices.

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**Please refer to the kit
insert for the Answers to
Study Questions**