4. PLASMOLYSIS

Background

Scientists, farmers, and gardeners rely on water potential to produce healthy plants. Plants only take in water when soil water potential is higher than plant cell water potential. During osmosis, water molecules diffuse down a water potential gradient from higher to lower water potential.

Water potential is influenced by two variables: solute amount and pressure. Solute concentration is usually the factor that is most responsible for determining if osmosis occurs. Solutes lower the water potential of a solution because *hydration shells* around solute particles make some water molecules less free to move, lowering the potential for water movement from that solution to another region. Distilled water has a water potential of zero; all solutions have a negative water potential.

Plant and animal cells respond similarly when surrounded by fluid hypertonic to the cell. Hypertonic solutions have a relatively high amount of solute, which creates a low water potential. A low water potential draws water out of cells. In plant cells, water loss is called *plasmolysis*. When plant cells lose water, cytosol volume decreases and the cell membrane pulls away from the cell wall. If a plant or animal cell loses too much water, the cell dies. Plasmolysis can be observed with a microscope.

Turgor pressure is a characteristic of plant cells but not animal cells. Plant cells are turgid in hypotonic environments. Animal cells burst, or *lyse*, in this condition. The high water potential of a hypotonic solution causes water to enter plant cells, increasing the volume of the cytosol. Turgor pressure results from the cell membrane and cytosol pushing against the cell wall. If plant cells experience plasmolysis, they lose turgor pressure—which is why a plant wilts in dry soil.

Driving Question

How can the water potential of plant cells be determined?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- Conductivity sensor
- Microscope, 400× magnification
- Microscope slides and cover slips (4)
- Graduated cylinder, 10-mL
- Test tubes, 1.5 cm × 15 cm (3)
- Stirring rod
- Plastic pipet or eye dropper
- Scalpel
- Three salt solutions of unknown concentration, several drops
- Red onion section
- Distilled water, 30 mL
- Tap water, several drops
- Paper towel

Safety

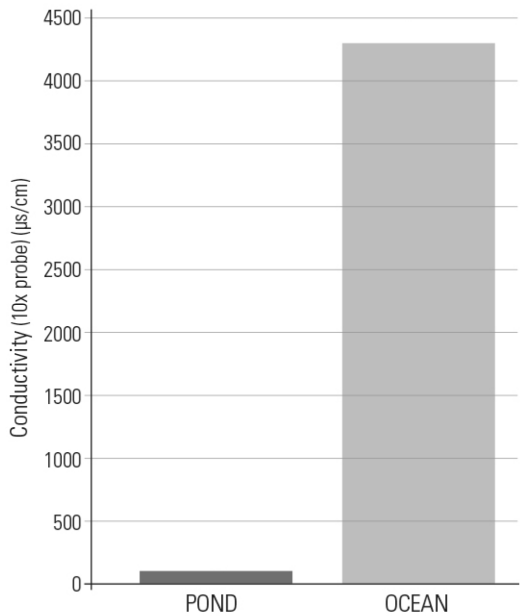
Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Use caution when cutting onion or vegetable samples with a scalpel.
- Take care not to break microscope slides. Tell your teacher if there is broken glass.
- Do not eat or drink any laboratory materials.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Refer to the bar graph to answer the following questions.



Note: Original pond water and ocean water samples were diluted to 10% solutions to accommodate the conductivity sensor range.

   a. Why is the conductivity of the two water samples different?

   b. Which water sample is most likely to cause plasmolysis in a plant cell? Explain the basis of your answer.

2. Prepare a wet mount of a very thin layer of red onion tissue. Use tap water as the liquid for this wet mount.

3. Prepare three additional microscope slides with onion tissue.

   a. For each of these slides, use one of the three "unknown" solutions (A, B, or C) as the liquid for the wet mount.

   b. Label the slides or place the slides on a labeled paper towel to keep track of which solution was used for each wet mount.

4. View the onion–water wet mount under the microscope. Select an area of the onion tissue that is thin and provides a good view of individual cells. Draw and record detailed observations; if possible, take a photograph of the cells.

5. View the wet mount prepared with Unknown A. Draw 1–3 cells and record differences you notice between this slide and the original one.

6. Observe the Unknown B and Unknown C slides. Compare and contrast the cells of these samples with previous samples and record detailed observations. Draw or photograph the cells.

7. The unknown solutions are all solutions of sodium chloride (NaCl). Based on your observations of the cells, which “unknown” solution has the highest salt concentration? Which has the lowest salt concentration? Explain the reasoning for your choices.

8. Fill a clean graduated cylinder with exactly 9.0 mL of distilled water. Use the pipet to add exactly 1.0 mL of Unknown A for a total cylinder volume of 10.0 mL. Stir to mix.

9. Pour the solution into a clean test tube. Label the test tube “A”.

10. Rinse the graduated cylinder, pipet or eye dropper, and stirring rod. Repeat Steps 9-11 for Unknown A and Unknown B.

11. Open the 4 ABI Plasmolysis lab file. Connect the conductivity sensor to your device.

    NOTE: If the lab file is not available, create a data table and switch to manual sampling mode to display the conductivity readings.

12. Begin data collection. Use the conductivity sensor to test the conductivity of each “unknown” solution. Rinse the sensor between solutions. Record the results of the conductivity test in a table. Multiply the sensor values by 10 to calculate the actual solution conductivity. Include the results in your table.

13. Do the results confirm your earlier ranking of the salt concentration in the solutions? Explain your answer.

Use the information below about water potential to answer the questions that follow.

The equation used to calculate water potential is

$$\Psi = \Psi_s + \Psi_p$$

A solution in an open container has a pressure potential Ψ_p of 0 bars, so in many situations this component of the equation can be left out, so that $\Psi = \Psi_s$.

The solute potential Ψ_s is equal to $-iCRT$ where

i is the ionization constant

C is the molar concentration

R is the pressure constant [0.0831 liter bar/(mole K)]

T is the temperature in K (273 + °C of the solution)

For distilled water, Ψ_s = 0 bars since there are no solutes.

14. Unknown A is a 0.12 M salt (NaCl) solution. Calculate the water potential Ψ of this solution at 21 °C. (In an open container, $\Psi_p = 0$.)

15. Refer to the water potential you calculated for Unknown A to answer the following.

    a. Is the water potential of a red onion cell greater than or less than the value you calculated for Unknown A? How do you know?

    b. How does the water potential of Unknown C compare to that of Unknown A? Support your claim with evidence and clear reasoning.

16. Adding distilled water to plasmolyzed cells can return the cells to a turgid state. (Test this for yourself if you have time!) Why don't plant cells burst when placed in a hypotonic environment, such as distilled water?

17. Yesterday your teacher placed celery stalks into distilled water and salt water. Record the initial and final mass of each celery stalk. Record observations of the stalks, comparing them to one another and to standard celery stalks you find in a grocery store. Use your understanding of osmosis and water potential to explain what occurred in each stalk overnight.

Design and Conduct an Experiment

A number of methods have been developed to experimentally determine the water potential of plant tissues. One method, the gravimetric technique, involves measuring the change in mass that occurs in tissue samples exposed to solutions of known concentration. For your experiment, devise a method to use the gravimetric technique to compare the water potentials of two or more types of plant tissue.

Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan. Then complete the Data Analysis and Synthesis Questions.

Design and Conduct an Experiment: Data Analysis

1. From your observations and your data:

    a. Explain the effect of the different sucrose solution concentrations on plant tissue samples.

    b. Determine the sucrose molarity at which each plant tissue sample would experience no change in mass (that is, there would be no net movement of water occurring.)

    c. Calculate the water potential for each plant tissue sample tested.

    NOTE: At equilibrium the pressure potential is assumed to be zero, since the solutions are in an open container and there is no net movement of water into or out of the cells.

2. Do the results of the experiment support or reject your hypothesis? Support your claim with evidence.

3. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?

4. Identify any new questions that have arisen as a result of your research.

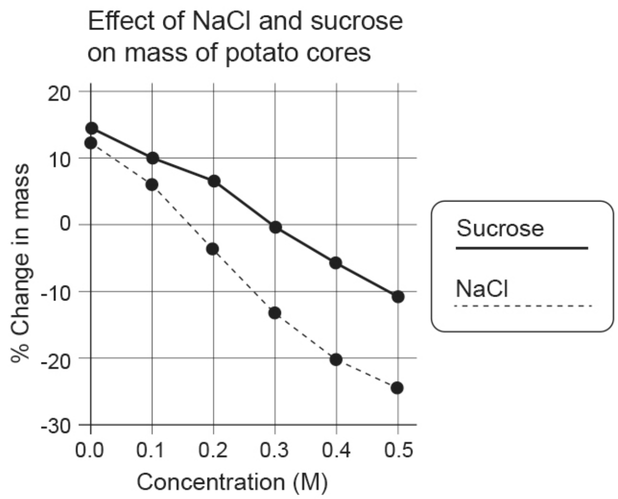
Synthesis Questions

1. Salt is used as a preservative to prolong the shelf life of foods without refrigeration. Describe how a salty environment would affect the cells of organisms, such as bacteria, that typically cause food spoilage.

2. Osmosis plays a role in regulating the opening and closing of stomata in leaves. Stomata are small openings through which a plant obtains the carbon dioxide molecules needed for photosynthesis. Guard cells are cells that are adjacent to stomata and change in shape to cause stomata to be open or closed. If guard cells swell due to osmosis, stomata open. It has been discovered that a high concentration of potassium ions (K^+) is needed for this to happen. Explain why an increase in K^+ concentration in guard cells causes the cells to swell.

3. Use the concept of "free energy" to explain why water molecules tend to move from an area of higher water potential to an area of lower water potential during osmosis.

4. Potato cores were placed in solutions with varying concentrations of sodium chloride (NaCl) and sucrose ($C_{12}H_{22}O_{11}$) for 24 hours. The percent change in mass of the cores in each solution was calculated and plotted on a graph.[1] Consider the equation for water potential and the properties of sodium chloride and sucrose as solutes, and explain the different results for the cores in the two solutions.



[1] Kosinski, R.J.; Osmosis and the Water Potential of Potato Tissue. Clemson University. Last update: October 2013. Retrieved February 6, 2014 from http://biology.clemson.edu/bpc/bp/lab/110/osmosis.htm.

NAME PERIOD DATE

Design and Conduct an Experiment Worksheet

A number of methods have been developed to experimentally determine the water potential of plant tissues. One method, the gravimetric technique, involves measuring the change in mass that occurs in tissue samples exposed to solutions of known concentration. For your experiment, devise a method to use the gravimetric technique to compare the water potentials of two or more types of plant tissue.

Develop and conduct your experiment using the following guide.

1. Consider the possible sources of plant tissue for this lab. List examples of tissues you might use.

2. Your teacher will provide you with a large volume of a 1.0 M sucrose solution. What range of diluted solutions do you think would be appropriate to use to determine the water potential of the plant samples?

3. Write a driving question: choose two or more plant tissues, or choose other solutions in addition to sucrose, and develop a testable question for your experiment.

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

6. Write a testable hypothesis (If…then…).

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

8. How many trials will be run for each experimental group? Justify your choice.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.