

DIFFUSION and OSMOSIS

Readings: Review pp. 105-109 in your text (POHS, 5th ed.).

Introduction

The lab discussion and exercises described below will illustrate the kinetic energy of molecules, diffusion, osmosis, osmolarity of cells, and pressure development in cells. The experiments will give you some understanding of how physical and chemical principles affect living systems. Each section in this exercise includes its own data analysis and write up directions.

LAB GOALS:

Following completion of the protocols below, you should be able to explain what happened in each experiment and to define and use the following terms: selectively permeable, solvent, solute, diffusion, osmosis, hypoosmotic, hyperosmotic, isoosmotic, osmotic pressure, osmolarity, Brownian movement, kinetic energy, plasmolysis, turgor pressure.

EXPERIMENTAL PROTOCOLS

NOTE: Begin Protocol 3 first. Follow directions in each protocol as to whether you will work individually or in pairs.

Protocol 1. KINETIC ENERGY OF MOLECULES

Introduction.

Here you will observe a phenomenon called "Brownian movement" by observing the random movement of particles in water. The movement of these particles comes about as water molecules collide with them, transferring some of the kinetic energy of the water molecules to the particles. This random movement of molecules is what causes diffusion to take place.

Procedure (work individually):

1. Place a drop of water on a slide.
2. Wet the tip of a dissecting needle and with it touch some of the dry carmine powder provided (carmine is a biological stain).
3. Add the carmine powder on the needle to the drop of water on the slide, mix gently, and place a cover slip over the suspension.
4. First, observe the carmine particles at low power on the compound microscope (4X objective). Then, increase magnification and observe the movements of:

(a) small particles, and (b) larger particles over periods of 2 to 5 minutes. Compare the movements of the two sizes of particles and note the directions in which they move.

Data analysis and write up:

1. Do small particles move more rapidly than large particles? Why?
2. Are the movements of the particles random or unidirectional?
3. Does the movement of the particles ever stop?
4. Are you actually observing molecular movement? Explain.
5. How can the movement being observed bring about diffusion?

Protocol 2. OSMOSIS IN LIVING SYSTEMS

Introduction.

All organisms must maintain an optimum internal osmotic environment. In this exercise you will investigate the osmotic behavior of plant tissues and cells and animal cells when placed in solutions of different concentrations. What happens to these cells when they are placed in hypoosmotic or hyperosmotic solutions?

A. Osmosis in plant tissue (potato)

For this exercise you will need: (1) a solution of sodium chloride (NaCl), (2) two glass staining dishes, and (3) two slices of potato, each at most 1 cm thick.

Procedure (work in pairs):

1. Dilute the 5 M stock solution of NaCl to 2 M and fill one dish half full.
2. Fill the other dish half full of distilled water.
3. Prepare the two potato slices, note their firmness and texture, and place one in each solution.
4. After 45 minutes note the firmness and texture of each potato slice again; if the two have the same firmness use a higher molarity NaCl solution (3 M).
5. After making your observations switch the potato slices; after another 45 minutes make the same observations as above.

Data analysis and write up:

1. How would you expect the potato slices to respond to the different solutions if they were living? Dead?
2. How did the two potato slices differ after the first part of the experiment?
3. How do you explain the difference?
4. How did they differ after the second part of the experiment?
5. How do you explain this difference?
6. Are the cells in potatoes living or dead?

B. Osmosis in plant cells (*Elodea* leaves)

For this exercise you will need: (1) three microscope slides labeled **A**, **B**, and **C**, (2) three pieces of *Elodea* leaves (*Elodea* is an aquatic flowering plant), and (3) solutions of two different concentrations (A and B).

Procedure (work individually):

1. Put 1 or 2 drops of the water in which the *Elodea* is maintained on **slide C**, place a small piece of an *Elodea* leaf (4-6 mm square) or the apical third of a young submerged leaf on the slide, and cover with a cover slip. Examine the cells closely and note their appearance; in particular, note the positions of the chloroplasts, which under normal conditions are at the outer surface of the cell.
2. Mount another leaf on **slide A**, using **solution A** instead of water from the *Elodea* container. Place the slide on the microscope and examine the cells for several minutes. Note and record any changes in cell appearance or in the positions of the chloroplasts. Changes do not always happen right away, so if you see none at first, put the slide aside and observe it every 5 minutes for about 30 minutes.
4. Repeat step 2 for **slide B**, using **solution B** (slide C is left unaltered as a control with which to compare the treated slides A and B).

Data analysis and write up:

1. Which solution is hypoosmotic to the plant cells? How did you arrive at this conclusion?
2. Which solution is hyperosmotic to the plant cells? How did you arrive at this conclusion?
3. What happens in plant cells in hypo- and hyperosmotic solutions?

C. Osmosis in animal cells (blood cells)

For this exercise you will need: (1) three microscope slides labeled **A**, **B**, and **C**, (2) dog or cat blood, (3) solutions of two different concentrations (A and B).

Procedure (work individually):

1. Place a drop of blood on **slide C**, add a cover slip, and observe the shapes of the red blood cells (save this slide for future reference).
2. Put a drop of **solution A** on **slide A** and add a cover slip. Place the slide on the microscope and carefully add a small drop of blood at the edge of the cover slip. The blood cells should be drawn under the cover slip; if they are not apply the edge of a paper towel to the opposite edge of the cover slip.

3. Watch the cells as they come into contact with **solution A** and for several additional minutes. Note the size, shape, and appearance of the blood cells and record your observations.
4. Repeat steps 2 and 3 with **solution B**, recording your observations each time.

Data analysis and write up:

1. Which solution is hypoosmotic to the blood cells? How did you arrive at this conclusion?
2. Which solution is hyperosmotic to the blood cells? How did you arrive at this conclusion?
3. What happens in animal cells in hypo- and hyperosmotic solutions?

Protocol 3. OSMOLARITY OF PLANT CELLS

Introduction.

In the following experiment you will estimate the osmolarity of potato tuber cells by measuring changes in weight. You will incubate pieces of potato tuber in sucrose solutions of known molarity. The object is to find the molarity at which the weight of the potato tissue does not change, indicating that there has been no net gain or loss of water. This molarity is an indirect measure of the osmolarity of the potato tuber.

For this exercise you will need : (1) sucrose solutions of eight different molarities (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 M), (2) eight 150 ml beakers, and (3) eight cylindrical pieces of potato.

Procedure (work in pairs):

1. Label the beakers with the eight sucrose molarities and place 100 ml of the corresponding sucrose solution in each.
2. From a potato, obtain cylinders of tissue with a sharp cork borer. Push the borer through the potato from the side, twisting it back and forth. When the borer is completely through the potato remove it and push the potato cylinder out. You will need 8 complete undamaged cylinders.
3. Check the lengths of the cylinders and trim them if necessary; they should be 5-6 cm long. Keep them covered in a petri dish until they are used. *From step 4 on, treat each sample individually!* Steps 4-6 should also be done quickly for greatest accuracy.

4. Zero the balance with a weighing dish or weighing paper on it. Blot all excess liquid from the outside of one potato cylinder by rolling it back and forth on a paper towel with your fingers.
5. Weigh the potato cylinder to the nearest 0.01 gram, and immediately place it in one of the solutions. Record the weight of the potato cylinder along with the molarity of the solution in which it was placed.
6. Repeat steps 4-5 for each of the remaining 7 potato cylinders (make sure the balance is zeroed before each weighing).
7. Leave the potato cylinders in the solutions 1.5 to 2 hours. *For greatest accuracy, the remaining steps (8-9) should be done quickly; assemble all needed items near the balance before beginning. Remove and weigh the cylinders in the same order in which they were initially placed into the solutions.*
8. Zero the balance with a weighing dish or weighing paper on it.
9. Remove the first potato cylinder and blot excess liquid from the outside of it *exactly as it was done the first time* (the potato cylinder should be the same "dryness" as before). Weigh the cylinder to the nearest 0.01 gram and record its weight.
10. Repeat steps 8-9 for the remaining 7 potato cylinders.

Data analysis and write up:

1. Calculate the percent change in weight of each potato cylinder using this formula: $\% \text{ change} = \text{weight change} / \text{initial weight} \times 100$. Be sure to note whether the weight changes were positive or negative.
2. On a piece of graph paper, plot percent change in weight as a function of the sucrose molarity.
3. Draw a curve to best fit the 8 data points. Use this curve to estimate the osmolarity of the potato tuber.

Answer the following questions:

1. What is osmolarity?
2. At what molarity does the curve cross the zero weight change line on the graph?
3. How can this information be used to determine the osmolarity of the potato tuber tissue?
4. What is the approximate osmolarity of potato tuber tissue?
5. What results would you expect if you used sodium chloride instead of sucrose for this experiment?

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