Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 34, 397-404, 2013

Osmosis, Lab Math, & Microscopes:An Inquiry Based Approach for Reviewing Basic Lab Skills and Concepts While Investigating Plasmolysis in *Elodea* Cells

Joanna R. Vondrasek

Piedmont Virginia Community College, Health & Life Sciences Division, 501 College Drive
Charlottesville VA 22902 USA
(jvondrasek@pvcc.edu)

In this inquiry-based lab suitable for cell biology or introductory biology, students design an experiment to determine the sodium chloride concentration that will cause plasmolysis in *Elodea canadensis* leaf cells. This lab engages students in experimental design using simple and familiar laboratory techniques, but it also serves as a review of cell structures, osmosis, and essential laboratory skills. Students prepare and observe living cells using microscopy and review laboratory math by making and diluting salt solutions. At the end of the activity, lab groups compare protocols and results. Several possible extensions to the lab are presented.

Keywords: osmosis, plamolysis, lab skills, plant cells

Introduction

Although microscope usage and laboratory math skills are covered in our Introductory Biology course, I find that many students entering my sophomore level cell biology course have poor microscope skills, and many are not proficient at making solutions or performing dilutions, skills critical to student success in upper-division laboratory courses. In order to conserve precious lab time and provide a meaningful, interesting lab activity to address these deficiencies, I turned a common directed lab activity on osmosis in plants into an inquiry based exercise in which students review basic lab skills, engage in experimental design, and discuss how subtle differences in protocol and experimental criteria can lead to variable results.

One of the more common introductory lab activities to address student understanding of cellular function, plant cell structure, and osmosis is a directed activity in which the leaf cells of the freshwater aquatic plant *Elodea canadensis* are observed under both hypotonic and very hypertonic conditions (see Perry et al., 2013 for an example of this laboratory exercise). In a typical directed protocol, students:

1. Make a wet-mount slide using a single *Elodea* leaf and a drop of tap water or the water in which the plants are growing (hypotonic environment in which the cells are turgid)
2. Observe the cells under control/baseline conditions using a compound light microscope (at various magnification levels).
3. Are given a high salt (NaCl) solution (usually between 10% and 20% w/v) and asked to repeat the procedure under hypertonic conditions to observe plasmolysis.
4. Repeat the procedure a third time using distilled or deionized water to demonstrate very hypotonic conditions (in some configurations of the activity)
5. Observe the plant cells, noting how cellular morphology changes between conditions.

As this lab is typically performed, students then sketch the cells in the different salinities, label visible cell structures, and answer a few questions regarding the physiological changes that have occurred with the addition of the salt solution.

When I have used a similar activity in my introductory biology class, the students are usually intrigued at how different the *Elodea* cells look in the control and hypertonic conditions, and, given a prior introduction to osmosis and membranes and plant cell structure, understand how water is moving across the cell membrane. They gain some experience using light microscopes and observing, sketching and labeling cells; however, as a completely directed exercise, the lab lacks any input from the student. The reagents are provided, the protocol is provided, and the student is just asked to complete the procedure and observe.

With the exact same supplies needed for the directed lab, plus a little more time, this lab is easily turned into an inquiry-based lab with the question, but not the protocol, provided

to the student. By engaging students in an inquiry-based lab as the first lab of the semester in a second-year cell biology course that requires them to review previously acquired lab skills, students quickly remember (or learn) how to make solutions and dilutions, how to make wet-mounts of tissue, and how to use a light microscope. With little guidance from the instructor, the students are given the opportunity to decide upon their own criteria for determining whether a cell has plasmolyzed and then use these criteria to determine the concentration of sodium chloride that triggers plasmolysis in *Elodea*.

Student Outline

Objectives:

- Review how to make solutions and perform dilutions
- Use a light microscope to examine plant cells
- Observe the effects of different salt concentrations on plant cell morphology
- Work with your lab group to design an experiment

Background

This lab is designed to give you practice making solutions and dilutions and using light microscopes to observe cells, which will be key skills for subsequent labs. After you make some stock salt solutions, you will design an experiment with your lab group to examine the effects of those salt solutions on the leaf cells of a freshwater aquatic plant, *Elodea canadensis*.

Part 1. Molarity and Dilutions

Concept Review 1: Solutions

Remember, the solute is the substance dissolved; the solvent is the liquid in which the solute is dissolved, and the resulting mixture is the solution. A one molar solution (1M) of a substance is one mole of the substance dissolved in a total volume of 1 liter (L).

Easy formula for determining molarity:

Mass (in grams) = Molarity * Volume (in **liters**) * molar mass

Example - The molar mass of **NaCl is 58.44**. Therefore, a 1 Molar solution (1 M) of NaCl has 58.44g NaCl dissolved in dH_2O to a total volume of 1 L.

Answer the following questions and check answers with your instructor before proceeding:

1. How much NaCl do you need to make 500mL of a 1.5 M NaCl solution?
2. How do you measure out the volume of water needed?
3. What sorts of equipment do you need?

Once you have checked your answers with your instructor, proceed to make 500mL of 1.5M NaCl using the materials provided on the back lab bench. (Glassware, NaCl, balance, weigh boats, stir bars, etc.).

Concept Review 2: Dilutions

Making dilutions is EASY! All you need to do is remember the formula $C_1V_1=C_2V_2$, where C=concentration (like molarity) and V=volume. The volumes and concentrations can be in any units, but both V_1 and V_2 must be in the same units and C_1 and C_2 must be in the same units.

Example - You are given a 5M stock solution of NaOH and asked to make 100mL of a 1M solution of NaOH. You need to determine how much of the 5M stock solution you need to make the lower concentration solution.
So, C_1 = 1M, V_1= 100mL, C_2=5M, V_2=?

To solve for V_2 → you will need 20mL of 5M stock solution diluted in 80mL of water to make 100mL of 1M NaOH.
Answer the following question and check answers with your instructor before proceeding:

1. Determine how to make 50mL of 1M NaCl using your "stock solution" from part 1 above. (Use the dilution formula to determine how much stock solution you need in 50mL.)

Once you have checked your answer with your instructor, proceed to make 50mL of 1M NaCl.

Make sure to label your salt solutions and keep them on your lab bench.

Part 2. Osmosis, Plant Cells, Plasmolysis and Microscopy

Concept Review 3: Osmosis and Plasmolysis

When a plant cell is **turgid**, the central vacuole of the cell is filled with water and the cell contents within the plasma membrane maintain cell shape by exerting outward pressure (**turgor pressure**) onto the cell wall. The inflexible cell wall exerts pressure back onto the plasma membrane and prevents further uptake of water (Freeman, 2011). When plant cells are placed in a hypertonic solution, they undergo **plasmolysis**, in which much of the water exits the cell via osmosis and the phospholipid-rich plasma membrane pulls inward. The relatively rigid cell wall comprised of cellulose, however, retains its shape. Initially you will make a slide of an *Elodea* leaf under baseline conditions (hypotonic). Then you will observe the leaf cells under very hypertonic and very hypotonic conditions.

PREDICT: Predict what will happen to plant cells when placed in baseline, very hypertonic and very hypotonic solutions.

Today you will be making a slide of an intact *Elodea* leaf and observing the leaf cells using the compound light microscope. You will be able to see the cell wall, plasma membrane and chloroplasts and possibly the vacuole (an absence of chloroplasts is a good indication of the rough outline of the vacuole).

Task 1. Making a wet mount of an Elodea leaf in various conditions

1. Remove one small leaf from the *Elodea* plant.
2. Place the leaf in the center of a glass microscope slide.
3. Take a plastic pipette and remove some of the water from the *Elodea* dish and place one drop on top of the leaf.
4. Carefully place a plastic cover-slip on top of the leaf, making sure to remove all air bubbles.
5. View the leaf under your microscope using all three magnifications and observe the usual appearance of the cells under normal osmotic conditions on high power and sketch a cell or two in your notebook.
6. Record your observations. What cell structures can you see? Neatly label any visible cell structures. Remember to record magnification and estimate the size of the cell and visible organelles if possible.
7. After observing, remove the slide from the stage.
8. Place a small piece of paper towel on the slide, adjacent to cover slip.
9. The paper towel will wick the water from beneath the cover slip.
10. Add three drops of 1M NaCl solution to the slide by placing 2-3 drops at the edge of the cover-slip and letting it wick under the cover-slip.
11. Wait 5 minutes.
12. Observe the slide again. Note any changes in appearance.
13. Repeat procedure with a fresh leaf using deionized water.

STOP: At this point we will discuss osmosis and plasmolysis as a group before moving on to the second part of the lab.

Task 2. Determining threshold for plasmolysis – an inquiry-based lab

A note on inquiry-based lab exercises: In scientific experiments the answers are not known ahead of time. Scientists have to devise appropriate protocols to test their hypotheses. Even if most or all of the procedures involved are standard techniques, scientists still have to determine what sorts of data they want to collect and how data will be interpreted. For most of the lab exercises during the semester, you will first complete a standard directed lab (where you are given a protocol and question), and then in the second portion of the lab you will ask a new question about the same system, devise a hypothesis and come up with a method to test your hypothesis.

MISSION: Your task is to determine what concentration of NaCl causes plasmolysis in Elodea leaf cells.

You have now seen what *Elodea* leaf cells look like when placed in a concentrated salt solution; but at what external NaCl concentration do the cells begin to lose significant amounts of water? Consider the following when designing your protocol:

- How will you determine when a cell has experienced plasmolysis? What will it look like?
- How will you determine the difference between plasmolyzed cells and flaccid cells?
- How long will you allow the cells to sit in the salt solution before making your observations
- How many leaf preparations will you make at each concentration?
- How will you account for variability in leaf size/shape?
- How will you record data?
- How will you present your results graphically?

Get ready for the experiment:

- Discuss your strategy with your lab partners
- Jot down a protocol in list form
- Make data recording tables

STOP: Show your protocol to your lab instructor. Once you've discussed your protocol with your instructor, complete the experiment with your lab group.

Post-lab Discussion and Analysis

Each lab group should prepare to share the following information with the rest of the class:

- What concentration of NaCl triggered plasmolysis (we will record all groups' estimates on the board)
- What your methods were for determining the point of plasmolysis

There is no formal lab write up for this lab, but you will need to **include the following** in your lab notebook

- Clear, repeatable protocol for your experiment – may be written in list format
- All data tables and graphs (label axes!) and explanatory captions
- A clear indication of what concentration of NaCl caused plasmolysis in your lab group's experiment
- Why you think different groups had varying estimates of concentration
- What physiological implications you think changing salinity in the water has for this plant and for other aquatic organisms
- What would have happened if you put an animal cell in hypo- and hyper-tonic conditions? Contrast with plant cells.

Notes for the Instructor

Biology Concepts

Instructors should ensure that students have had an introduction to the topics of semi-permeable membranes, osmosis, turgor pressure, and basic plant cell structure. This can be provided through classroom lecture/discussion, pre-lab reading, or pre-lab lecture.

Lab Skills

Students should have previously made molar solutions and used light microscopes. It is possible to introduce dilution making in this lab, but students will be more effective if dilutions have been introduced previously. I use this lab as the first lab of the semester for a sophomore-level cell biology course, so I know that they have at least had exposure to all the lab concepts in the introductory biology lab sequence (where we do a directed version of this lab very similar to Task 1 and separately introduce solution making and dilutions). This lab can be modified to fit into an introductory biology course as long as the basic skills involved have previously been introduced. I do not recommend this lab for students who have no experience using the microscope.

Guiding the Lab Activity

After a review of skills and concepts at the beginning of the lab, instructors should give students (working in lab groups) time and freedom to generate their own methods for evaluating the question posed. They should be encouraged to think about what the criteria are for determining whether a cell (or whole field of view) of *Elodea* has plasmolyzed. As a note, different groups will likely differ significantly on how they define plasmolysis. When I presented this lab to participants at the ABLE conference (about thirty biologists), seven different groups came up with seven different criteria.

Examples of criteria for plasmolysis generated by PVCC students or ABLE participants:

- When **half** the cells in a field of view have visible pulling in of the cell membrane (ABLE participants)
- When the **first** cell in a field of view has visible pulling in of the cell membrane (PVCC students and ABLE participants)
- When **all the cells** in a field of view have visible pulling in of the cell membrane (PVCC students)
- When "most" the cells in a field of view have visible pulling in of the cell membrane (I challenged this group to define "most" more carefully) (PVCC students)
- When cytoplasmic streaming and movement of the chloroplasts stop (PVCC students)
- When the cells look "clumpy" (as defined by the group – students came up with a mutually agreed upon definition and did a multiple observer test at my prompting to see if "clumpy" was a repeatable measure – it was) (PVCC students)

Common student difficulties:

- Diluting their stock solution in their stock solution bottle.
- Making such large volumes of diluted solution that they run out of stock solution.
- Students often predict that plant cells will burst under very hypotonic conditions (deionized or distilled water). It is usually necessary to review how vacuoles and the cell wall function to prevent bursting, as would happen in animal cells in similar conditions.
- Reusing the same leaf over and over while starting with a high salt concentration and working to a lower salt concentration. **

** Depending on the initial salinity, it is possible for cells to recover from some degree of plasmolysis; however, this method is not reliable for determining the tipping point, and in general students decide to use new leaves for each concentration increment or to start at a low concentration and work to higher concentrations using the same leaf. Again, differing estimates of concentration generally result from the new leaf vs. reused leaf method, probably because of a dilution effect from the existing solution on the leaf when stepping up to higher concentrations. New leaf vs. reused leaf also makes for an interesting discussion of methods.

Post-lab discussion

A key to teaching this lab effectively is to let the student groups devise their own methods and criteria, even if the methods seem poor or unreliable, but to then have a class discussion of results (and criteria and methods) at the end of the lab period. Each lab group should record their data (concentration in M of plasmolysis point) for collective viewing and the class data should be discussed. Often, as is evident in Table 1, the concentration of NaCl (or other salts) that students determine to cause plasmolysis will vary quite a bit depending on how they conducted their experiment (what the plasmolysis criteria were, how long they left the salt solution on the leaf before viewing).

Table 1. Student lab group estimates of plasmolysis point for *Elodea* cells in various salt solutions.

Salt	Lower estimates	Upper Estimates
NaCl	0.3 M	0.9 M
$CaCl_2$	0.2 M	0.5 M
KCl	0.5 M	0.8 M

The instructor should be prepared to guide students through a discussion regarding repeatable criteria for determining plasmolysis, and how different criteria would affect results. Initially, students are confused when other groups post their results and they do not match their own. However, as each group stands up and explains their criteria and methods, the rest of the class will often nod in understanding - since it will

sometimes be apparent immediately why different groups ended up with different estimates. This was particularly evident one semester when one group chose "all the cells" and another group chose "one cell" in a field of view (on different magnifications) to determine the point of plasmolysis.

Evaluation

Although I do not traditionally have students write up a formal lab report for this exercise, it would work especially well as the first formal lab write up in a semester because the techniques are not difficult or unfamiliar, and instruction can focus on scientific paper format and analysis since students will not have to struggle with new procedures. I do have students keep a lab notebook and record protocols, record and analyze results, and answer a few questions that I include at the end of the handout. I give a pre-lab quiz the following week in which students are asked to solve some molarity and dilution problems and answer a few questions regarding osmosis and plant cell structure.

Variations / Extensions to the Lab

This lab offers the possibility of several additional activities to be completed after the initial investigation:

Other solutes: Students can repeat the procedure using a different salt (a second salt can be done in the same week if students need minimal preparation on solution making). I have previously used both KCl and $CaCl_2$ in laboratory sections. As long as the same criteria are used to define plasmolysis, students can investigate why different salts trigger plasmolysis at different concentrations. Sugar solutions can also be tested – comparing mono- and disaccharides (glucose vs. sucrose), for example.

Other organisms: Salt water aquarium plants can be tested with the same salt and compared to Elodea. Freshwater protists such as Paramecium or Amoeba (lacking cell walls) can be examined and compared to plant cells (live protists are inexpensive and available from Carolina Biological).

Environmental applications: commercial ice melting preparations can be tested, and students can speculate how run-off of these chemicals from roadways might affect plant life - great for a winter time lab in colder climates.

Background research: The literature on plasmolysis in plants goes back at least 100 years, and students might enjoy reading some of the early work on plant cells and comparing methods with more recent investigations.

Materials Required:

- *Elodea canadensis* plants – one healthy 6-7cm sprig for each lab group.

Plants can be purchased from Carolina Biological Supply (Catalog # 162112 - $21.50 for a pack of 25 plants in 2012 catalog). *Elodea* can also be purchased at many pet stores that carry aquarium supplies. *Elodea* can be purchased in advance and maintained in the lab in a large glass bowl or in an aquarium with adequate natural or artificial light. Make sure students have access to the water in which the *Elodea* is maintained, as this is the baseline solution to which they will be comparing their high salt solutions.

- Dry NaCl, KCl, $CaCl_2$ or other solutes
- Compound light microscopes – one or two per group of 4 students
- Glass microscope slides
- Plastic or glass cover-slips
- Graduated cylinders
- Volumetric flasks
- Beakers / test tubes (*I have previously provided small 50mL beakers for dilutions, but the ABLE participants requested test tubes and test tube racks to make small volume dilutions)
- Lab balance
- Lab tape / markers
- Weigh boats and spatulas
- Deionized water
- Stir plates and stir bars
- Pipettes or micropipettes (disposable 1.5mL plastic pipettes work well for dropping salt solutions onto slides, but this is also a good opportunity for students to review micropipette use)

Note on Materials

Given the self-directed nature of this lab, I suggest putting lab supplies in a common area rather than per lab bench so lab groups can decide which supplies they need.

Acknowledgments

I thank Sean Miller, who evaluated salt tolerance of *Elodea* for his sophomore thesis project, the PVCC Cell Biology classes of 2010 and 2011, Rosalyn Koontz and Jennifer Scott for laboratory support, and the participants of the mini-workshop at the ABLE meeting in Chapel Hill in 2012 for helpful suggestions and a demonstration of how 30 biologists would approach this lab as students.

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About the Author

Joanna Vondrasek received her B.A. in Biology from Swarthmore College and her Ph.D. in Biology from the University of North Carolina at Chapel Hill. She is currently an Associate Professor of Biology at Piedmont Virginia Community College in Charlottesville, VA where she teaches Introductory Biology, Cell Biology and Genetics.

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Citing This Article

Vondrasek, J.R. 2013. Osmosis, Lab Math, & Microscopes:An Inquiry Based Approach for Reviewing Basic Lab Skills and Concepts while Investigating Plasmolysis in *Elodea* Cells. Pages 397-404 in *Tested Studies for Laboratory Teaching,* Volume 34 (K. McMahon, Editor). Proceedings of the 34th Conference of the Association for Biology Laboratory Education (ABLE), 499 pages. http://www.ableweb.org/volumes/vol-34/?art=42.