

Lab 3: Diffusion and Osmosis

Molecules in solution tend to move down the concentration gradient. In this lab exercise, we will use a simple system to measure the rate of movement of water molecules in the presence of different gradients. We will use Excel to analyze the data, determine the relationship between concentration gradient and rate of osmosis, and predict the gradient from an observed rate of osmosis.

Objectives

When you have successfully completed this lab, you should be able to:

1. Explain the principles of diffusion and osmosis.
2. Create a standard curve using known sucrose concentrations and use this standard curve to determine the concentration of an unknown.
3. Write a lab report explaining your work.
4. Use trendlines and their equations to think about the relationship between two variables.

IMPORTANT: A portion of your grade for this laboratory depends on your ability to determine the concentration of an unknown, and you will utilize skills you learned during the first two labs. Be sure to think about what you will do before the lab period begins and to be careful as you work; your unknown experiment must be carried out during this lab period.

Before lab, please purchase colored pencils. A package with five colors would be ideal, but you will need at least one red and one blue

Introduction

The plasma membrane of the cell is selectively permeable, which means that it allows the passage of some substances while it prevents the passage of others. Cells constantly move molecules across the plasma membrane, into and out of the cytoplasm. Some molecules are actively transported across the membrane, a process which requires transport proteins and energy input. Active transport occurs against a concentration gradient. In contrast to this active transport, diffusion and osmosis describe the movement of molecules along the concentration gradient in a passive process that does not require cellular energy.

A **gradient** is simply the condition that exists when something is distributed unevenly. One example of a gradient is a temperature gradient. It is usually warmer in the south and cooler in the north. The temperature is distributed unevenly. Another example of a gradient is the difference in altitude between a lake high in the mountains and the ocean below. This example is a useful way to think about the potential energy inherent in a gradient. The water in the lake will tend to flow towards the ocean, not the other way around. This tendency to flow from high to low is analogous to the tendency of dissolved molecules to move from an area of high concentration to an area of low concentration. We all know that energy in the form of hydroelectric power can be harvested as water flows downhill. Later in the course, we will discuss how energy is harvested from a concentration gradient in cellular organelles such as chloroplasts and mitochondria.

Diffusion is the movement of dissolved molecules from a region of high concentration to a region of low concentration. This process tends to spread the molecules out. Keep in mind that diffusion occurs even in the absence of a membrane.

Osmosis is the diffusion of water molecules across a selectively permeable membrane. Selectively permeable membranes allow some substances, including water, to pass freely through the membrane, but block the passage of others. As in diffusion, the water molecules move from a solution with a higher concentration of water to a solution with a lower concentration of water.

In this laboratory exercise, you will learn about some of the principles of diffusion and osmosis. Along the way, you will design and carry out a simple experiment. The data you collect during this experiment will, when properly analyzed and displayed, allow you to determine the concentration of an unknown sucrose solution.

Procedures

Section A - Diffusion

The movement of substances across the plasma membrane is important to the survival of the cell. Certain substances must enter the cell to serve as raw materials and to provide an energy source. Likewise, waste products must move out of the cell. The molecules in liquid water are in constant motion, colliding with each other and with molecules of dissolved substances. This kinetic molecular motion allows diffusion to occur.

Think about a shoebox with twenty marbles all stacked against the left side. These marbles represent dissolved molecules, with a high concentration at the left end of the box and a low (zero) concentration elsewhere in the box. A concentration gradient exists - high at the left end of the box and low everywhere else. Now think about shaking the box. This is where the constant motion of molecules comes in. Marbles will tend to move down the concentration gradient, that is from left to right in the above example. When you open the box, the marbles are randomly distributed. The concentration of marbles has evened out, and a concentration gradient no longer exists.

Temperature can affect the rate of diffusion. To see this, place a drop of food coloring into a small beaker of room temperature water. Count the number of seconds required for the dye to be evenly distributed throughout the beaker. Now repeat the experiment using a beaker of hot water. Allow the water to boil, remove from the heat, let stand 30 seconds, then add the food coloring and record the time required for even distribution. Do the particles move faster or slower in the hot water? Why? (Be sure to include your description of this activity and answer these questions in your lab report.)

Section B - Osmosis

Like any molecule, water can be present in high or low concentrations. While it may at first seem counterintuitive to imagine one solution with a higher concentration of water than another solution, this actually happens all the time. Think about a 5% salt solution. By weight this solution is 5% salt and 95% water. Imagine that this solution was placed across a semipermeable membrane from a distilled (0% salt and 100% water) water solution. Water molecules would move down their concentration gradient from the high concentration to the low concentration, i.e., from the 100% solution to the 95% solution, but the membrane would prevent the salt molecules from moving. (See Figure 8.12 on page 147 in your text.)

The following protocol will allow you to determine the rate at which distilled water crosses a semipermeable membrane enclosing a sucrose solution. Think about how you would use this method to determine the concentration of a sucrose solution of unknown concentration. Remember that a rate has units such as miles per hour or grams per minute. In this experiment, you will determine the rate of osmosis by determining the slope of a line.

1. Each lab group should find 6 lengths of dialysis tubing, each 5 inches long, at their table. Dialysis tubing is a semipermeable membrane that is selectively porous and allows some molecules to pass through it. You also have several beakers which you should label using the red wax marker in your lab drawer. Do not mark glassware on the white frosted marking area of the beaker. Marks made there with wax pencil DO NOT come off.

2. Soften the tubing by soaking each piece in a beaker of distilled water for a few minutes. The ends of the tubes can now be opened by rubbing them gently between your fingers.

3. Seal one end of the tube by folding it over and tying it off very tightly with dental floss.

4. Using a separate funnel and graduated cylinder for each solution, pour about 20 ml of each of the following solutions into the open end of each separate bag: distilled water, 1% sucrose, 5% sucrose, 10% sucrose, 15% sucrose and 20% sucrose. Tie off the open end of each bag very tightly, but leave about an inch of empty tubing above the top of the solution. This extra space is called “head space” and is essential because water will enter the bags during the experiment. Note that all solutions look the same once they are in the bag. Keep careful track of which bag is which.

5. Rinse the bags with distilled water, gently blot dry, and record the mass of each bag using the electronic balance. Leaking bags will give useless data, so be sure that your bag is not leaking before you start. Handle the bags carefully to ensure that you don't damage them in the process of blotting and weighing. Do not prepare all of your bags at once and leave them sitting on the table top while you tie and fill the rest. Fill one, tie it, weigh it and place it in distilled water immediately. Use the same electronic balance for each weighing.

6. Immerse each bag in a beaker of distilled water. (Is the volume of water in the beaker a fixed variable?) Blot and reweigh each bag every ten minutes for at least forty minutes. Record the masses in the table on the next page. As you collect your data, construct a single graph with scatter plots of the mass of each bag versus time. Use your colored pencils, with a different color representing each bag. Can you guess at the trendlines for these plots?

Make sure you complete the plot, including axis labels. Answer the questions on the following page, and get your instructor's signature before leaving the laboratory.

Later, you will graph these data in Excel and determine the equation for each trendline. The slopes of these lines are the rate at which the water moves into the bags. Recall that the slope of a line is the change in y divided by change in x or dy/dx or $(y_1 - y_2 / x_1 - x_2)$. You should produce six lines, one for each percentage of sucrose you used.

QUESTION: What should you probably be doing with your unknown at this time?

Experiment with an Unknown

Determine the concentration of your unknown sucrose solution. You should run your experiment at the same time as you are running the experiment above.

Record the number of your Unknown Solution here:

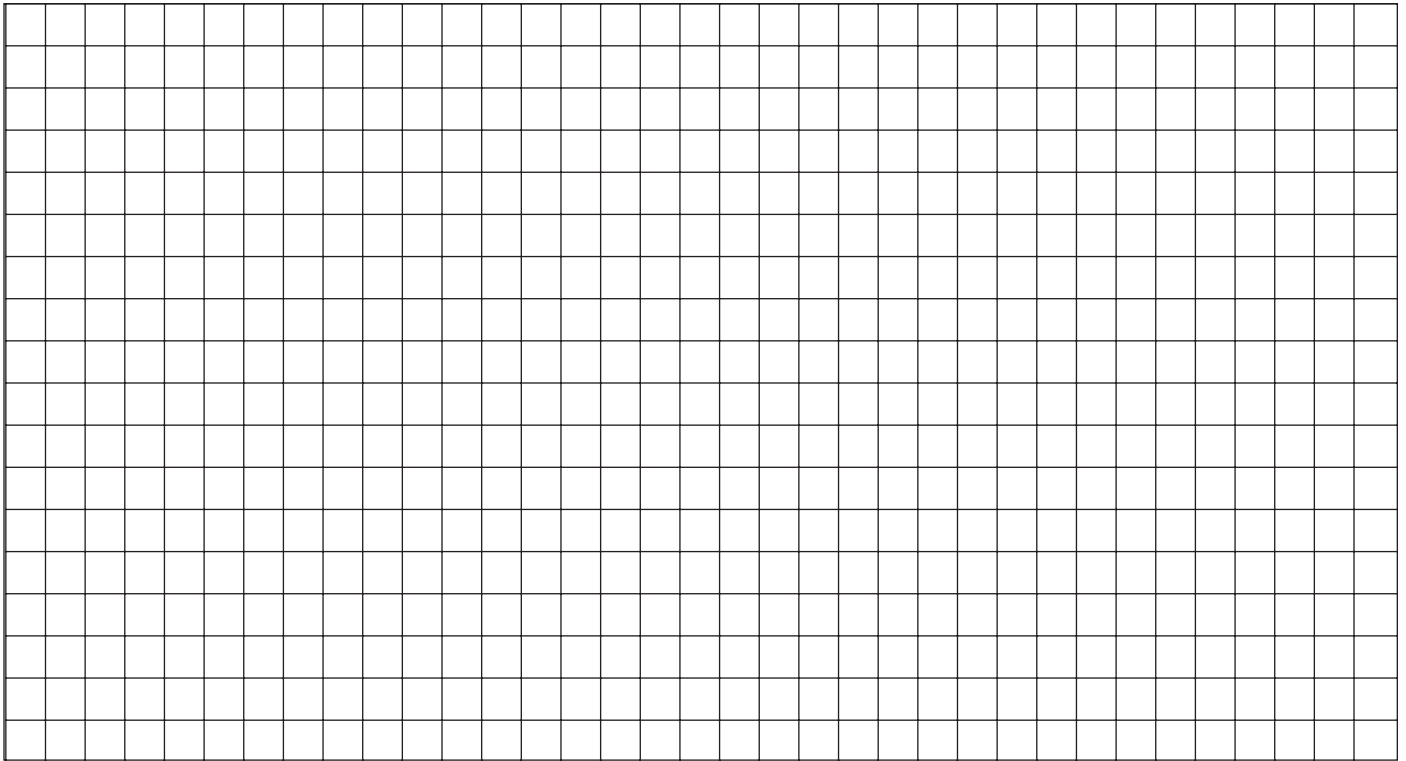
Make sure you refer to it in your lab report.

Section C - Data Analysis

After taking a few data points, you have probably noticed that the mass of each of your dialysis bags tends to increase with time. Use the graph paper on the next page to plot the data during the experiment, and think about the following questions.

1. Does the bag increase in mass at a constant rate?
2. What factors might introduce uncertainty into the measurement of this rate?
3. Is the rate of mass increase related to the concentration (%) of sucrose inside the bag?
4. How can you determine the rate at which water enters each bag?
5. Are these data good enough to complete your assignment, or should all of the members of your lab group make an appointment to repeat the exercise?

Table 1 Mass of Dialysis Bags at Timed Intervals					
Contents of Dialysis Bags	0 minutes	10 minutes	20 minutes	30 minutes	40 minutes
0 % Sucrose (Distilled Water)					
1 % Sucrose					
5 % Sucrose					
10 % Sucrose					
15 % Sucrose					
20 % Sucrose					



From these data, what is your first estimation of the concentration of sucrose in your unknown?

Are these data good enough to be sure of your unknown concentration?

Obtain your instructor's signature here after completing your graph and answering the questions.

After leaving the laboratory, use Excel to help you examine your osmosis data. Keep in mind that the rate of osmosis means the amount of water that enters the bag per time interval. This rate depends on the concentration of sucrose; the higher the concentration of sucrose, the faster water enters the bag. You can determine the rate by finding the trendline slope expressed in grams per minute.

Then, use the rates from the known sucrose concentrations to graphically determine the relationship between concentration of sucrose and rate of osmosis. Use this relationship to construct a standard curve which can be used to determine the percentage for your unknown sucrose concentration.

Section D - Dialysis

Dialysis involves the selective separation of materials by differential diffusion across a membrane. The artificial kidney machine, used for patients whose kidneys have impaired function, works on this principle. The patient's blood is passed outside of the body and into a special dialysis chamber bounded by a selectively permeable membrane. The chamber is surrounded by a dialysis buffer. Small molecular waste products pass out of the blood into the buffer, and the concentration of waste products equilibrates across the membrane. Large molecules and red blood cells are retained in the dialysis chamber. The blood is then returned to the body with a reduced concentration of waste products. As an example of differential diffusion, observe the demonstration with a dialysis bag containing starch solution, and liquid outside the bag containing iodine. Iodine stains starch a dark blue.

For your lab report: Note the location of the blue color - is it inside of the bag or outside? What gradients are present? Which diffusion occurred? Why? How did that lead to the results you saw?

Section E - Reporting your Experiment

This week, you are to write a lab report, which will be due at the beginning of class in two weeks. Remember, this is not creative writing. Your goal is to explain your work clearly and concisely.

There are many ways to write a scientific report, but all scientific writing has some specific rules. Reports should be organized in labeled sections: Introduction, Methods, Results and Discussion. After you finish the exercise, you should begin writing your introduction and methods sections and begin developing the graphs for your results section. The sooner you write the results section, the sooner you will find out if you need to repeat your experiment. A complete discussion of how to write a successful lab report can be found in the Appendix of your laboratory manual.

Your **introduction** should, in your own words, explain the background for your experiment. Then, near the end of the introduction, explain your question or hypothesis. You should use your textbook, your lab manual and outside sources to help you write this section. For the diffusion and osmosis lab report, the introduction should include an explanation of the principles of diffusion and osmosis and how these influenced your experiment. Please cite any outside references in the text of your report; use the author's last name and the page number (Smith, p 612). The complete reference should be found at the end of the report in a bibliography section. (Example: Smith, Mary. "The Bacterial Cell Wall." *J. Bacteriology* **118**: 612.)

The **methods** section should explain, briefly, but completely, how you did your experiments. Tell your reader about your experimental design. What are your independent and dependent variables? The methods section should be written in the past tense, passive voice. For example "Cells were grown for three days.", or "Dialysis tubes were filled with sucrose solutions."

The **results** section is where you report your data. This is where you place tables and graphs, along with written explanations of what is in them. For this experiment, your results section should include your data, slope calculations and percent sucrose of the unknown solution. Be sure to include the number of the unknown solution you used.

A **discussion** section contains interpretation of your data, explaining how it fits into the greater body of knowledge. Once you have completed your

experiments, you are in a position to draw conclusions based on your results. Specifically, you must attempt to interpret your results in the context of the specific hypothesis that you set out to address. Some of the issues to consider when making conclusions include:

What did you expect to find, and why?

How did your results compare with those expected?

How might you explain unexpected results?

How might you test these potential explanations?

Should your hypothesis be modified?

Before you finish your lab report, consult Appendix B, page B1 to see the kind of evaluation sheet that will be used to grade your work.

Lab Assignment - Exercise 3

This assignment is to be handed in at the beginning of lab in two weeks (before your quiz). Each student must complete this assignment individually.

Your lab report must be typed, doubled spaced and you must use a 12 point font. Number your pages and label each section. Do not use a title page, simply place the title of your report at the top of the first page. If you tend to have trouble with your grammar and sentence structure, please ask for assistance at the Writing Center. Keep in mind that the Writing Center will not fix your report for you, but they will help you to learn how to fix it yourself. Your lab group worked together to gather data for this experiment, which means that four of you will have identical data. However, each person is responsible for his/her own written presentation and analysis. This means each student must create graphs and tables on his or her own. **Do not paraphrase or use uncited text from your book or manual. Answer all questions on your own. Copying will not be tolerated.**

WHAT DOES THIS MEAN? Turning in others' work as your own is a violation of the Honor Code, and will be punished harshly. Two students who work together and submit identical graphs, tables, or answers to questions are **BOTH** cheating.

To ensure that students are turning in original work, all text materials from this assignment must be submitted electronically to www.turnitin.com. This procedure usually takes five to ten minutes and you must have access to the internet. Your assignment will not be graded, and may be counted late until we receive your originality report.

1. Point your web browser to <http://www.turnitin.com/>
2. Click on User Login, and then click on **New User** (unless you already have an account at turnitin.com).
3. Enter your UE email address and choose a password (case-sensitive). Remember your password. You will need it for the rest of your time at UE. You may want to write your password here: _____ . Click the **next** button to enter your name and address. When you are finished, return to the login page and login as a student. You only need to register once. From now on, just login as a student.
4. Click on **join new class**. Join Biology 107. The course ID is **1158566**, and the enrollment password is **meiosis**. You only need to join Biology 107 once. From now on, just click on your Biology 107 link.
5. To submit a paper, click on the **turn it in!** button. Select an assignment (in this case, choose Diffusion and Osmosis). Paste all of your text for this assignment into the text box or select a file to upload. You do not need to submit graphs or tables - they disappear when you paste. Press submit, and you are done.

Let's recap what is due in two weeks:

1. Your osmosis lab report with Introduction, Methods and Results and Discussion sections, in your portfolio, complete with corrected quizzes.

In the Results section, you must indicate which unknown solution you used and its calculated percent sucrose.

2. A description of the temperature experiment (page 31). What happened and what does it mean?
3. A description of the dialysis demonstration, which should include answers to the questions on page 34.
4. Answers to the four questions on the following page.
5. All text for this assignment submitted to www.turnitin.com on the day you hand in the material in class.

Questions:

1. A scientist places a pH 5 solution on one side of a membrane and a pH 8 solution on the other. What gradients exist in this situation? Would the pH be closer to 5 or closer to 8 after extended diffusion? How does this compare to the role of gradients and energy in the process of oxidative phosphorylation (text Chapter 9)?
2. A horticulturalist knows that high concentrations of fertilizer salts in the soil can actually hurt plants. Explain what this has to do with osmosis.
3. A biologist working with cultured cells uses distilled water in place of serum. When he looks for the cells, he sees only debris. What happened to his cells?
4. You used a straight line to determine your rate of osmosis. Why? Would this linear relationship between mass and time continue over long periods of time? Why or why not? Include in your answer a sketch of the relationship of mass and time over long time periods.

Before you turn in your assignment, go back and re-read your instructions - have you forgotten anything? Does your report include a sentence like "The concentration of unknown # ___ is ___%"? Have you made every effort to ensure that your concentration is accurate?

Have you labeled, titled and properly assembled your portfolio? See page 2 of the syllabus for portfolio instructions. Make sure your corrected quizzes are present in your portfolio.

Did you use the Appendix material and sample evaluation sheets to help you write your report?

Did you run the spell checker on your text AND have you proof read it before printing your final copy?

Did you number your pages and place your name at the beginning of the paper?

Have you submitted all text material for this assignment to www.turnitin.com?

Do you understand what osmosis means? Could you explain diffusion to an accounting major who lives next door to you in the dormitory? Could you explain it on a quiz?

Now you are ready to turn in your portfolio. It is due when lab begins, before your quiz.
