Purpose of lab:
- Practice application of the concepts of diffusion and osmosis.
- Practice designing a controlled experiment.
- Reinforce methods of statistical analysis and graphing
- Reinforce interpretation of results

CONCEPTS

Diffusion is the net movement of molecules of a substance from a region of their higher concentration to a region of their lower concentration. Net movement means there are more molecules moving in one direction than in the opposite direction.

Opening a bottle of perfume in a room will result in the gradual diffusion of the perfume from the region of higher concentration (the bottle) out into the room. Diffusion will continue until the perfume has a more or less uniform concentration throughout the bottle and room.

Solutions. A solution is composed of a solvent and a solute. A solvent is a medium in which one or more substances dissolve. In this laboratory the solvent is always water. A solute is a substance dissolved in a solvent. The concentrations of solvent and solute in a solution are inversely related. You can visualize this principle by imagining two identical graduated cylinders to which you first add different amounts of salt, then fill with water. You would have the situation seen in Figure 1. Fill in the blanks with the concentrations of salt and water in both cylinders.

Figure 1. Effect of the amount of solute on the amount of water in a solution.
From your work in Figure 1 you see that the % solute and the % water in a solution always total ____%. So a 5% sugar solution, for example, is 5% sugar and ____% H₂O.

**Diffusion Through Membranes.** A **differentially permeable membrane** is one that some molecules can pass through, while other molecules cannot. In this laboratory exercise we will use membranes with tiny holes in them that let small molecules like H₂O and salt (NaCl) pass through, but not larger molecules like sugars (e.g. C₆H₁₂O₆ and C₁₂H₂₂O₁₁).

**Osmosis** is the diffusion of a **solvent** through a differentially permeable membrane. In biological systems, the solvent will usually be water. Osmosis will occur whenever the water concentrations are different on either side of a differentially permeable membrane. Remember that the water concentrations are determined by the solute concentrations (Figure 1).

**PRACTICE APPLYING THE CONCEPTS**

Now you have all the information you need for solving problems dealing with diffusion across differentially permeable membranes. Let's work through some examples.

**Problem 1 / Figure 2A.** Imagine the situation shown in Figure 2A. A bag made of a differentially permeable membrane that contains a 5% sugar solution is placed in a beaker containing a 1% sugar solution. To deduce what should happen, complete the following problem-solving steps.

**Problem solving steps:**
1. In the space provided, write down what is given: the % of solute inside bag A in the blank provided and the % of solute outside the bag in the space provided. Deduce and write down the solvent concentrations in both places. (Reread the Solutions section above if unsure.)

2. Apply the definition of differentially permeable membrane: Can the solvent go through the membrane? _________________ Can the solute go through the membrane? _________________ (If unsure, consult the discussion of differentially permeable membranes above.)

3. Apply the definition of diffusion: For each substance that can go through the membrane, draw an arrow going through the membrane to show the net direction of diffusion of that substance. (If unsure, review the concept of diffusion).

4. Deduce the result of osmosis. Using the arrows that you drew, deduce whether the balloon should shrink, swell, or remain the same. Write your prediction in the space beside the figure.
Problem 2 / Figure 2B. Repeat steps 1-4 to predict what should happen in 2B if a bag with a 5% sugar solution is placed in a beaker containing 10% sugar solution.

Problem 3 / Figure 2C. Repeat steps 1-4 to predict what will happen in 2C if a bag with a 5% sugar solution is placed in a beaker containing 5% sugar solution.
Problem 4. The cells of fish that live in fresh water contain more solute than does the water in the lake or stream that surrounds the fish. A cell membrane is differentially permeable, like the membranes described above. As in Problems 1-3 above, draw a picture representing the situation and apply the same problem solving steps. Will water diffuse into or out of the cells of the fish? __________

Explain how you arrived at your answer: __________________________________________

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EXPERIMENT ON THE RATE OF OSMOSIS

I. Introduction

You saw in the practice problems that a cell either shrinks or swells if the concentrations of water inside and outside a cell are not equivalent. Either situation (shrinking or swelling) could be disastrous for an organism. Nevertheless, many organisms are able to live in solutions that are stronger or weaker than the solutions inside them by working (i.e., expending energy) to counteract the loss or gain of water.

To prevent cells from bursting, a freshwater fish must pump water out of its body as fast as the water enters. Osmosis, (i.e., the diffusion of water into or out of cells), requires no input of energy from the fish, but moving water against the direction of diffusion takes work – like bailing a leaky boat. Of course, the faster the water enters, the harder the fish (or fisherman!) must work to pump it out.

Thus, the rate of osmosis is critical to life, and organisms have adaptations to regulate the rate of osmosis to suit their needs. To imagine how organisms could adapt, we might first ask “What affects the rate of osmosis?”

One influence on the rate of osmosis could be the concentrations of the solutions inside and outside of the cell. If the difference in the concentrations are large, then osmosis might be faster. If the differences are small, then osmosis might be slower. We could make a hypothesis about the effect the difference in concentrations has on osmosis.

Hypothesis: The greater the difference between the concentration of water inside a cell and the concentration outside a cell, the faster the rate of osmosis.

This hypothesis could be wrong. Maybe the greater concentration difference will NOT lead to a faster rate of osmosis. What is an alternative hypothesis, i.e., what is another possible way the concentration difference could affect the rate of osmosis?
II. Methods

To test this hypothesis, we will make bags out of a synthetic material that contains microscopic holes. Your instructor will demonstrate how to fill the bags and suspend them in beakers of distilled water. The holes in the membrane are large enough to allow passage of small molecules, like water, but not of larger molecules, like sucrose. If water diffuses into or out of the bags, the bags will get lighter or heavier, so we can measure the rate of osmosis by seeing how fast the bags change weight.

You will have tap water, as well as solutions of 20% sucrose and 60% sucrose available. With these substances, and the dialysis bags, your class will design an experiment to test your hypothesis.

In preparation for the design, describe or explain these parts of your experiment. These should be part of any well-planned experiment.

**Independent variables** are the different treatments you will compare. This is the variable you are actually studying or manipulating. These values are chosen before the experiment starts. Refer to your hypothesis if you are unsure of what it is you are comparing. Include a control treatment, where you use the same methods as in the others, but expect no change.

**Dependent variables** will have values that depend on the independent variable’s values. The dependent value is what you will measure. Define what you will measure and exactly how you will measure it.

**Variables that should be held constant**. What things need to be kept the same for every treatment you measure? What will you do to make sure they are constant? The only thing that should choose to vary is the independent variable.

**Predictions (i.e. deductions)** Deduce what should happen in this particular experiment, if each hypothesis is true. That is, if the hypothesis is right, how should the different treatments compare in weight gain? If your alternative hypothesis is right, how should the weight gain of the treatments compare?
Replicate your experiment. Make a sample size of at least 3 of all your treatments. Three is still not a great sample size, but it's better than a single sample, which could give erroneous results and we'd never know it.

Data table. Draw a data table on paper to record the data you will get from your experiment.

As part of the plan for your experiment, make diagrams similar to those in Figure 2 to show your experimental set-up.

III. Results

Put data in your data table as you collect it. After you have collected all your measurements, calculate the means and standard error for each treatment.

Graph the data. (Think about what type of graph should be used with this type of data.) Graph the means and standard errors for each treatment.

A margin of error template is available in class and at the Biology Department's Statistics web site: [www.radford.edu/~biol-web/stats.html](http://www.radford.edu/~biol-web/stats.html). Go to the section on Margin of Error. Study the example for entering data in the spreadsheet template. Open the template and enter your data as instructed.
**IV. Conclusions and discussion**

Here you say if the results support the hypothesis, the alternative hypothesis or neither. First you need to use the margin of error statistic to conclude if the differences among the treatments are significant or not. (Refer to your 'Guide to Statistics Used in Biology' to make sure you understand how to use and interpret the 'Margin of Error Statistic.') Study the example and apply the same logic and language to your results. Did we get the differences among treatments predicted by our hypothesis or by the alternative hypothesis? Which hypothesis was supported? Explain how your data, especially the 'margin of error' statistic, support that hypothesis.

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Often, we can see new, unanticipated discoveries in our results, which should be discussed. For example, look at the graphed line for each of your treatments. Does the rate of diffusion in each treatment increase, decrease, or stay the same over the course of the experiment? Explain your answer. Note that the rate (weight change per unit of time) is reflected in the slope or steepness of the line.

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Now explain why we should get these results. To help, start by thinking about what happens to the concentration of the solution in the bags during the experiment. Does concentration gradient in each treatment increase, decrease, or stay the same over the course of the experiment? Explain why the curves in your graph seem reasonable.

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