

OSMOSIS

INTRODUCTION

Probably everyone has observed that a small amount of perfume will spread rapidly in a room, even without air currents carrying it. Likewise, a drop of food coloring in a glass of water will spread through the water and eventually create an even coloration throughout.

This phenomenon, the even spreading of particles within a medium, is called diffusion. It is the result of the random motion of the molecules or particles involved. The process of diffusion obeys the Law of Mass Action which states: *there will be a net movement of molecules from an area of higher concentration to an area of lower concentration until the distribution of molecules within the system is equal.*

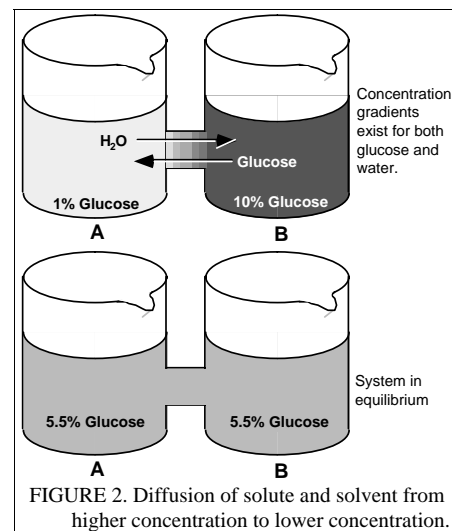
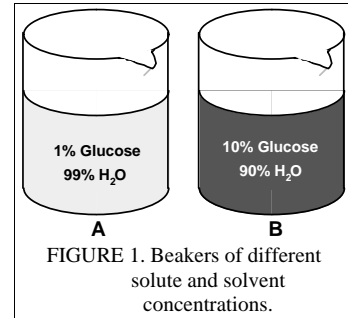
The process of diffusion plays an important role in the movement and distribution of materials in living systems. At the cellular level, gas exchange, absorption of many nutrients, waste elimination and intracellular transport are dependent upon diffusion.

The closely related phenomenon of osmosis may be defined in terms of diffusion. Osmosis is the diffusion of water through a semi-permeable membrane in response to a concentration gradient.

To understand these two processes and their differences, consider the situation in Figure 1: beaker A contains a solution of 1% glucose and beaker B contains a solution of 10% glucose. Obviously, beaker B contains a more concentrated solution of solute (glucose in this case). But if one considers the concentration of water (solvent) in the two beakers, one would have to say that beaker A is more concentrated in water (99% water in A as opposed to 90% water in B).

If the two beakers are joined by a tube as in Figure 2, the Law of Mass Action predicts a net movement of each molecular species (water and glucose) from an area of higher concentration to areas of lower concentration until the concentration of glucose and water in both beakers is equal (Fig. 2).

The movement of glucose and water in this example is due to the molecules obeying the *Law of Mass Action*. When there is no longer a net movement of molecules from one beaker to another, we say the system is in equilibrium. This doesn't mean that molecular movement has stopped. Rather, it means the movement of glucose from beaker B to beaker A is equal to its movement in the opposite direction. This is because a concentration gradient no longer exists. The same reasoning applies to the movement of water molecules.



In Figure 3 a membrane or filter which allows free passage of water molecules but prevents the movement of glucose molecules is inserted between the beakers. Thus, the glucose molecules cannot respond to the Law of Mass Action and move down their concentration gradient.

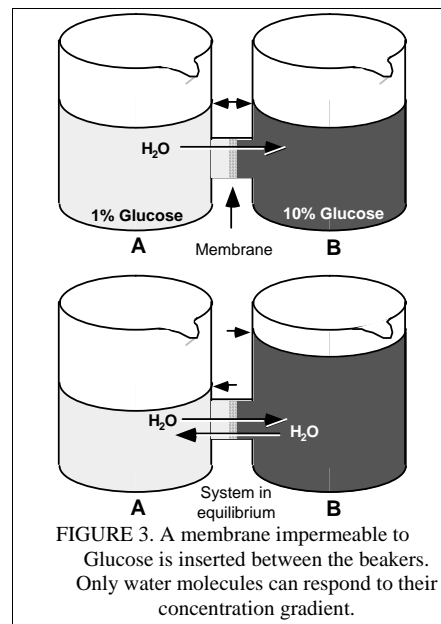
Since the filter does not present a barrier to the water molecules, they will move down their concentration gradient (99% → 90%). Because there is a net movement of water from beaker A to beaker B, the volume of A decreases while that of B increases (Fig. 3). This is the process of Osmosis: a net movement of water through a semi-permeable membrane (filter) in response to a concentration gradient.

Theoretically, osmosis should continue as long as a concentration gradient for water exists. Examination of the system in Figure 3 shows, however, that the net water movement into beaker B creates a higher fluid pressure in that beaker. This pressure tends to force water molecules back into chamber A.

Initially, when the levels of solutions in each column are equal, both chambers are exerting equal fluid pressure against the semipermeable membrane. As osmosis proceeds, a pressure gradient is created equal to the weight of the water moved into chamber B. It is this pressure gradient which tends to force water molecules back into beaker A.

Equilibrium is established when the net movement of water molecules into beaker B in response to the Concentration Gradient (osmosis) is equal to the net movement of water in the reverse direction in response to the pressure gradient. In other words, you observe a net movement of water from beaker A to Beaker B which gradually decreases the concentration gradient. At the same time, the increase in volume in beaker B gradually increases the pressure gradient which tends to force the water molecules back into beaker A. At equilibrium the flow of water in each direction is equal.

The process of osmosis has profound effects on living organisms. Cells are enclosed by a plasma membrane which is freely permeable to water, but selectively permeable to most other molecules. Cells contain a weak solution of dissolved proteins, carbohydrates and ions which are not freely permeable.



STRATEGY FOR MEASURING OSMOSIS

In this exercise, you will make bags that have walls of a semi-permeable material (dialysis tubing). You will place NaCl solutions of different strengths into the bags and then place the bags in a beaker of pure water. The walls of the bags are impermeable to NaCl molecules, but do allow water to move through. You will follow the movement of water by weighing the bags. If a bag loses weight during the course of the experiment, you know it is losing water. If it gains weight, it is gaining water.

MEASURING OSMOSIS

MAKE THE NAACL DILUTIONS

1. Keep all solutions away from the computer!
2. Set up and label 5 test tubes in the rack. Labels should be: 1.5; 1.0; 0.5; 0.25; H₂O.
3. Using the 1.5M stock NaCl solution, make 15ml of the following dilutions: 1.0M; 0.5M; 0.25M. Note that this is not a dilution series. Use the $C_1 \times V_1 = C_2 \times V_2$ relationship to make dilutions.

4. Place 15ml of the 1.5M stock in the tube labeled 1.5.
5. Obtain five strips of the dialysis tubing and prepare them by moistening and tying off one end. It is very important that the ends be tied off to prevent the flow of water. To do this, twist the end and double it over before tying off.
6. Label each bag with a small piece of tape folded back on itself on the end of one of the strings. Labels should be: 1.5; 1.0; 0.5; 0.25; H₂O.
7. Add approximately 750ml of deionized water (DI H₂O) to a 1000 ml beaker.
8. Pour 15ml of the following solutions into the appropriately labeled bag: 1.5M; 1.0M; 0.5M; 0.25M; and deionized water. Squeeze any air out of the bags and securely tie off the end of the bag and gently place on a paper towel on your bench. There must be room left in the bag for expansion.

WEIGH THE BAGS AT 10 MINUTE INTERVALS

Table 1. Osmosis Data					
		Time (Minutes)			
Conc. Grad.		0	10	20	30
0.00 M	Measured Weight				
	Cumulative Weight	0			
0.25 M	Measured Weight				
	Cumulative Weight	0			
0.50 M	Measured Weight				
	Cumulative Weight	0			
1.00 M	Measured Weight				
	Cumulative Weight	0			
1.50 M	Measured Weight				
	Cumulative Weight	0			

9. When all the bags are filled weigh each bag and place it in the beaker of water. This is the initial weight at time zero for each bag.
10. Record your data in Table 1.
11. Be sure to keep track of the order in which you weigh the bags, and use the same order in subsequent weighings.
12. Repeat the weighing process for each bag at exactly 10 minute intervals over 30 minutes. You should end up with 4 weights for each bag. Record each weight in the appropriate "Measured Weight" row in Table 1.
13. After the last measurement, puncture each bag and flush the sugar solution down the sink. Dispose of the empty bags in the trash.
14. Be sure to rinse and dry the weighing pan from the balance and clean up any spilled sugar solution.

ANALYSIS OF OSMOSIS DATA

Each bag in the beaker of water represents a different concentration gradient of water across a semi-permeable membrane. For simplicity, the solute concentration gradients will be used to describe each. Thus, the 0.5M NaCl solution bag in the beaker of water is said to have a 0.5M concentration gradient.

Weight change in the bags represents the gain or loss of water. By examining your data, you should be able to determine whether or not osmosis occurred and in which direction the water moved.

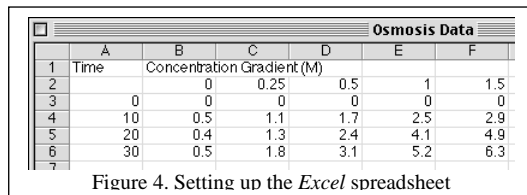
The bag containing water serves as a control. The change in weight observed in it is due to experimental error. One can expect small fluctuations in either direction due to water adhering to the bag or inconsistencies in weighing the bags.

CALCULATE THE CUMULATIVE WEIGHTS

1. The "Cumulative Weight" rows in Table 1 are for showing the weight the bags accumulate over the 30 minute experimental period. Note that at time 0, the accumulated weight is 0 because the bag hasn't had a chance to gain or lose water.
2. To calculate the "Cumulative Weights," subtract the "Measured Weight" at time 0 from the "Measured Weight" of time 10, 20 and 30. For example, if the "Measured Weight" at time 0 is 17 g, and the "Measured Weight" at time 20 is 19 g, the "Cumulative Weight" for time 20 is 2 grams (the weight accumulated over the 20 minute period is 2 grams).
3. Calculate the cumulative weights for your all bags and time samples in Table 2 and enter the values in the appropriate spaces in Table 1.

MAKE A SPREADSHEET OF THE CUMULATIVE WEIGHTS

1. Set up an *Excel* Workbook with the first sheet showing the measurement time intervals in column 1 and the concentration gradients in row 2 as in Figure 4.
2. Enter the cumulative weights in the appropriate data cells.
3. Notice that as time passes the weight of the bags changes. The change in weight is due to the bag gaining or losing water. One gram of water is equal to 1 ml in volume.



	A	B	C	D	E	F
1	Time	Concentration Gradient (M)				
2		0	0.25	0.5	1	1.5
3	0	0	0	0	0	0
4	10	0.5	1.1	1.7	2.5	2.9
5	20	0.4	1.3	2.4	4.1	4.9
6	30	0.5	1.8	3.1	5.2	6.3
7						

Figure 4. Setting up the *Excel* spreadsheet

MAKE A GRAPH SHOWING THE RELATIONSHIP BETWEEN TIME AND WEIGHT IN THE BAGS

1. The two variables for these data are concentration gradient and the passage of time. The weight of the bag does not determine how much time has elapsed, but rather, the amount of elapsed time determines weight change in the bag. For this reason, time is considered the independent variable and weight change the dependent variable in this relationship. Remember, the independent variable is plotted on the x-axis and the dependent on the y-axis.
2. Note the way the data are arranged in Figure 4. The X data (independent variable) are in the first column, with the weights of each of the bags arranged in succeeding columns. Cells in row 2 contain the identifiers (labels) for each bag. For reasons explained later, it's important to leave the cell above the X data blank for now.
3. Click and drag to select cells A2 : F6.
4. Click on the "Chart Wizard" tool and select the "XY (Scatter)" chart type. From the "Chart sub-type" menu, select the chart with no line connections. Click on the "Next > " button to go to step 2.

5. *Excel* assumes that the data sets are arranged in rows because the selected data are in more columns than rows. This is not the case. The data sets are in columns, so click on the "Columns" button in the Step 2 Window as in Figure 6.

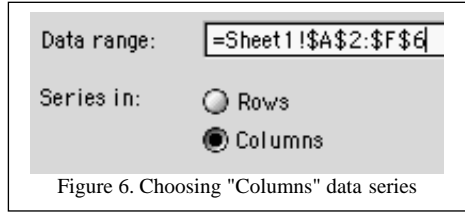


Figure 6. Choosing "Columns" data series

6. Since the first cell in column 1 of the selected data (cell A2) is empty, *Excel* makes two other important assumptions: 1) the data in the first column are the X data; and 2) those in the first selected row (row 2) are the identifiers ("Labels") for the Y data sets.
7. Click on the "Next >" button to go on to step 3.
8. In the "Step 3" window, type in the chart title, and the labels for the X and Y axis. Make sure the legend is showing.
9. Click on the "Next >" button to go on to step 4.
10. In the "Step 4" window, place the chart "As a new sheet" and click on the "Finish" button.
11. Add linear trend lines. Don't forget to remove the gray background.
12. Note how the weight change in each bag (concentration gradient) is represented by a separate line. By checking the legend, you can see that the bags gaining the most weight have the highest concentration gradient. All of the lines and the accompanying legend make this a rather complex graph. The next section shows you how to simplify it.

CALCULATE THE RATE OF OSMOSIS

1. The rate of osmosis, in grams of water per minute, may be calculated by dividing the weight gained in the bag by the time period.
2. You could calculate the rate for any of the time periods, but a good expression is the rate for the entire 30 minute period (divide the "Cumulative Weight" at 30 minutes by 30).
3. *Excel* can make this job easier. In cell A7, type in "Rate" as in Figure 8.
4. Now you will enter a formula in cell B7 that will take the cumulative weight at 30 minutes (cell B6) and divide it by 30 (cell A6).
5. To do this, click in cell B7, and type in "=". You should see the "=" appear in the cell and in the Formula Bar. *Excel* is now ready to accept a formula in this cell.
6. Click on cell B6. You should now see "=B6" in the cell and Formula Bar.
7. Now type in a "/" and click on cell A6. You should now see "=B6/A6" in the cell and formula bar as in Figure 8. You have just told *Excel* that the contents of cell B7 are equal to cell B6 divided by cell A6.

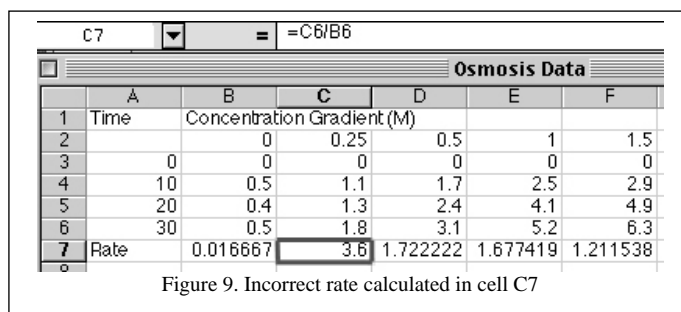
	A	B	C
1	Time	Concentration Gradient	
2		0	0.25
3	0	0	0
4	10	0.5	1.1
5	20	0.4	1.3
6	30	0.5	1.8
7	Rate	=B6/A6	

Figure 8. Entering a formula in cell B7

8. Press the enter key and note how calculated rate appears in cell B7, but "=B6/A6" still appears in the Formula Bar (that's why they call it the Formula Bar).
9. You want to do a similar calculation for cells C7 - F7. You could type the formula into each cell, or you could copy and paste the formula using the "Edit" pull down menu. However, *Excel* provides a shortcut for doing this.
10. Make sure cell B7 is selected. Locate the tiny box in the lower right corner of cell B7, click on it and drag to cell F7.
11. When you release the mouse, you should see the rate calculations in each cell. However, close inspection reveals that these are not the correct rates (Fig. 9).

12. Click in cell C7 and note what formula is shown in the Formula Bar. It is C6/B6. You are not dividing the cumulative weight gain by 30, but rather by the cumulative weight shown in column B.

13. Unless you instruct it otherwise, *Excel* assumes that you want all formulas to be relative to the defined cell. That is, the formula you entered in cell B7 told *Excel* to go up one cell (B6) and take that value, then up one cell and to the left one cell (A6) and use that value to divide into the first.

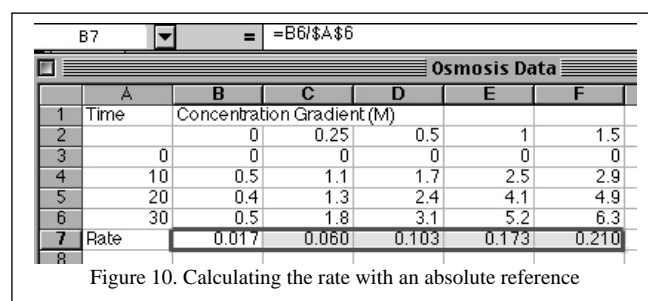


14. To fix your spreadsheet, and make all of the rate calculations divided by cell A6, you must make the reference to cell A6 "absolute." This is done by placing a "\$" in front of the row and column that is to be absolute. The absolute reference to cell A6 would be \$A\$6.

15. First, remove the incorrect formulas by selecting cells C7 - F7 and choosing "Clear Contents" from the "Edit" pull down menu.

16. Click in cell B7 to select it. In the Formula Bar, you should see the formula "B6/A6."

17. Click in front of the "A" in the Formula Bar so that you see the cursor bar flashing in front of the "A." Now type a "\$." Move the cursor to in front of the "6," and type in another "\$." The entry in the formula bar should now read "=B6/\$A\$6" as in Figure 10.



18. A "keyboard shortcut" for making an absolute reference to a cell is to click on the cell in the formula bar (between the "A" and "6" in Figure 9) and press the "Ctrl" and "T" keys at the same time.

19. With cell B7 selected, and the Formula Bar reading "B6/\$A\$6," press the "Enter" key. Next locate the tiny box in the lower right corner of cell B7 (Fig. 10), click on it and drag to cell F7.

20. When you release the mouse, you should see the correct rates appear in cells C7 - F7 (Fig. 10).

MAKE A GRAPH SHOWING HOW THE CONCENTRATION GRADIENT AFFECTS THE RATE OF OSMOSIS

1. In the previous graph, you showed the relationship between time and osmosis for each concentration gradient. In this graph, you want to show the relationship between concentration gradient and the rate of osmosis.

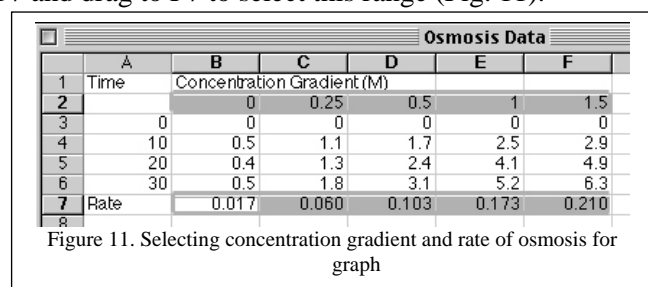
2. To do this, click on cell B2 and drag to F2 to select B2 - F2 as shown in Figure 11.

3. While holding down the Ctrl Key click on cell B7 and drag to F7 to select this range (Fig. 11).

4. Click on the "Chart Wizard" tool and make a graph of the selected data. Select "XY Scatter" and a line connecting the points.

5. In this case, the data are in rows, so make sure this option is selected in step 2.

6. In step 3, think of a snappy title for the graph and type it in. Label the X axis "Concentration Gradient (M)" and the Y axis "Rate of



Osmosis (g/min).” Also in this step, make sure that the "Show legend" box is not checked (you don't need a legend because there is only one data set).

7. Make formatting adjustments to graph until it looks good and expresses the relationship well.
8. Add trend lines and remove connected lines. Save and make a printout of the graph.

QUESTIONS FOR YOUR REPORT

1. Present an abstract of your work. Your abstract should include a brief description (1/2 page) of 1) what you set out to learn, 2) what procedures you performed, and 3) a summary of your data or discoveries.
2. Present a printout of your team's spreadsheet that shows the osmosis data for this experiment. It should include the rate of osmosis calculations.
3. Present a printout of the graph showing the relationship between time and the weight change in each of the dialysis bags.
4. Explain the purpose of the dialysis bag containing pure water. Did yours gain or lose weight during the experiment. If so explain.
5. Present a printout of the graph showing the relationship between concentration gradient and the rate of osmosis.
6. Using the appropriate graph as an example, describe the relationship between concentration gradient and the rate of osmosis.
7. Given enough time, you would observe that the rate of osmosis in each bag would begin to decrease, even though the bag is not full. Explain.
8. Pretend your lab partner made a bag that became rigid and stopped gaining weight at the 20 minute mark. What experimental error(s) did your partner do to cause this?
9. Why do grocery stores constantly spray water on their leafy vegetables in the produce section? Use principles of osmosis in your answer.
10. Give a specific example of how an organism adapts to changes osmotic pressure.