**LABORATORY 1: DIFFUSION AND OSMOSIS**

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| **OVERVIEW** | In this laboratory, you will investigate the processes of diffusion and osmosis in a model membrane system. You will also investigate the effect of solute concentration on water potential as it relates to living plant tissues. |
| **OBJECTIVES** | **Before doing this laboratory you should understand**:  \* the mechanisms of diffusion and osmosis and their importance to cells  \* the effects of solute size and concentration gradients on diffusion across selectively permeable membranes  \* the effects of a selectively permeable membrane on diffusion and osmosis between two solutions separated by the membrane  \* the concept of water potential  \* the relationship between solute concentration and pressure potential and the water potential of a solution  \* the concept of molarity and its relationship to osmotic concentration  **After doing this laboratory you should be able to:**  \* measure the water potential of a solution in a controlled experiment  \* determine the osmotic concentration of living tissue or an unknwn solution from experimental data  \* describe the effects of water gain or loss in animal and plant cells  \* relate osmotic potential to solute concentration and water potential |
| **INTRODUCTION** | Many aspects of the life of a cell depend on the fact that atoms and molecules have kinetic energy and are constantly in motion. This kinetic energy causes molecules to bump into each other and move in new directions. One result of this molecular motion is the process of diffusion.  **Diffusion** is the random movement of molecules from an area of higher concentration of those molecules to an area of lower concentration. For example, if one were to open a bottle of hydrogen sulfide (H2S has the odor of rotten eggs) in one corner of the room, it would not be long before someone in the opposite corner would perceive the smell of rotten eggs. The bottle contains a higher concentration of H2S molecules than the room does and, therefore, the H2S gas diffuses from the area of higher concentration to the area of lower concentration. Eventually a dynamic equilibrium will be reached; the concentration of H2S will be approximately equal throughout the room and no net movement of H2S will occur from one area to the other.  **Osmosis** is a special case of diffusion. Osmosis is the diffusion of water through a selectively permeable membrane (a membrane that allows for diffusion of certain solutes and water) from a region of higher water potential to a region of lower water potential. Water potential is the measure of free energy of water in a solution.  Diffusion and osmosis do not entirely explain the movement of ions or molecules into and out of cells. One property of a living system is active transport. This process uses energy from ATP to move substances through the cell membrane. Active transport usually moves substances against a concentration gradient, from regions of low concentration of that substance into regions of higher concentration. |
| **EXERCISE 1A: Diffusion** | In this experiment, you will measure diffusion of small molecules through dialysis tubing, an example of a selectively permeable membrane. Small solute molecules and water molecules can move freely through a selectively permeable membrane, but larger molecules will pass through more slowly, or perhaps not at all. The movement of a solute through a selectively permeable membrane is called **dialysis**. The size of the minute pores in the dialysis tubing determines which substances can pass through the membrane.  A solution of glucose and starch will be placed inside a bag of dialysis tubing. Distilled water will be placed in a beaker, outside the dialysis bag. After 30 minutes have passed, the solution inside the dialysis tubing and the solution in the beaker will be tested for glucose and starch. The presence of glucose will be tested with Benedict's solution or Testape. The presence of starch will be tested with Lugol's solution (Iodine Potassium-Iodide or IKI). |
| **Procedure** | 1. Obtain a 30-cm piece of 2.5-cm dialysis tubing that has been soaking in water. Tie off one end of the tubing to form a bag. To open the other end of the bag, rub the ends between you fingers until the edges separate.  2. Place 15 mL of 15% glucose/1% starch solution in the bag. Tie off the other end of the bag, leaving sufficient space for the expansion of the contents in the bag. Record the color of the solution in Table1.1.  3. Test the 15% glucose/1% starch solution for the presence of glucose. Your teacher may have you do a Benedict's test or use glucose Testape. Record the results in Table 1.1.  4. Fill a 250-mL beaker or cup two-thirds full with distilled water. Add approximately 4 mL of Lugol's solution to the distilled water and record the color of the solution in Table 1.1. Test this solution for glucose and record the results in Table 1.1.  5. Immerse the bag in the beaker of solution.  6. Allow your set-up to stand for approximately 30 minutes or until you see a distinct color change in the bag or in the beaker. Record the final color of the solution in the bag and of the solution in the beaker, in Table 1.1.  7. Test the liquid in the beaker and in the bag for the presence of glucose. Record the results in Table 1.1. |

**Table 1.1**

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|  | **Solution Color** | **Presence of Glucose** |  |

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| --- | --- | --- | --- | --- | --- |
|  | **Initial Contents** | **Initial** | **Final** | **Initial** | **Final** |

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| **Bag** | **15% glucose & 1% starch** |  |  |  |  |
| **Beaker** | **H2O + IKI** |  |  |  |  |

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| **Analysis of Results** | 1. Which substance(s) are entering the bag and which are leaving the bag? What experimental evidence supports your answer?  2. Explain the results you obtained. Include the concentration differences and membrane pore size in your discussion.  3. **Quantitative data** uses numbers to measure observed changes. How could this experiment be modified so that quantitative data could be collected to show that water diffused into the dialysis bag?  4. Based on your observations, rank the following by relative size, beginning with the smallest: glucose molecules, water molecules, IKI molecules, membrane pores, starch molecules.  5. What results would you expect if the experiment started with a glucose and IKI solution inside the bag and only starch and water outside? Why? |
| **EXERCISE 1B: Osmosis** | In this experiment you will use dialysis tubing to investigate the relationship between solute concentration and the movement of water through a selectively permeable membrane by the process of osmosis.  When two solutions have the same concentration of solutes, they are said to be **isotonic** to each other (iso means same, -ton means condition, -ic means pertaining to). If the two solutions are separated by a selectively permeable membrane, water will move between the two solutions, but there will be no net change in the amount of water in either solution.  If two solutions differ in the concentration of solutes that each has, the one with more solute is **hypertonic** to the one with less solute (hyper means over, more than). The solution that has less solute is **hypotonic** to the one with more solute (hypos means under or less than). These words can only be used to compare solutions.  Now consider two solutions separated by a selectively permeable membrane. The solutions that is hypertonic to the other must have more solute and therefore less water. At standard atmospheric pressure, the water potential of the hypertonic solution is less than the water potential of the hypotonic solution, so the net movement of water will be from the hypotonic solution into the hypertonic solution. |
| **Procedure** | 1. Obtain six 30-cm strips of presoaked dialysis tubing.  2. Tie a knot in one end of each piece of dialysis tubing to form six bags. Pour approximately 25 mL of each of the following solutions into separate bags:  a) Distilled water  b) 0.2 M sucrose  c) 0.4 M sucrose  d) 0.6 M sucrose  e) 0.8 M sucrose  f) 1.0 M sucrose  Remove most of the air from each bag by drawing the dialysis bag between two fingers. Tie off the other end of the bag. Leave sufficient space for the expansion of the contents in the bag. (The solution should fill only about one-third to one-half of the piece of tubing).  3. Rinse each bag gently with distilled water to remove any sucrose spilled during filling.  4. Carefully blot the outside of each bag and record in Table 1.2 the initial mass of each bag, expressed in grams.  5. Fill six 250-mL beakers or cups two-thirds full with distilled water.  6. Immerse each bag in one of the beakers of distilled water and label the beaker to indicate the molarity of the solution in the dialysis bag. *Be sure to completely submerge each bag.*  7. Let them stand for 30 minutes.  8. At the end of 30 minutes remove the bags from the water. Carefully blot and determine the mass of each bag.  9. Record your group's data in Table 1.2. Obtain data from the other lab groups in your class to complete Table 1.3: Class Data. |

**Table 1.2: Dialysis Bag Results: Individual Data**

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| **Contents in Dialysis Bag** | **Initial Mass** | **Final Mass** | **Mass Difference** | **Percent Change in Mass \*** |
| **a) Distilled Water** |  |  |  |  |
| **b) 0.2 M** |  |  |  |  |
| **c) 0.4 M** |  |  |  |  |
| **d) 0.6 M** |  |  |  |  |
| **e) 0.8 M** |  |  |  |  |
| **f) 1.0 M** |  |  |  |  |

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| **\* To calculate:**  Percent Change in Mass = (Final Mass - Initial Mass / Initial Mass) x 100 |

**Table 1.3: Dialysis Bag Results: Class Data**

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|  | **Percent Change in Mass of Dialysis Bags** |  |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Group 1** | **Group 2** | **Group 3** | **Group 4** | **Group 5** | **Group 6** | **Group 7** | **Total** | **Class Average** |
| **Distilled Water** |  |  |  |  |  |  |  |  |  |
| **0.2 M** |  |  |  |  |  |  |  |  |  |
| **0.4 M** |  |  |  |  |  |  |  |  |  |
| **0.6 M** |  |  |  |  |  |  |  |  |  |
| **0.8 M** |  |  |  |  |  |  |  |  |  |
| **1.0 M** |  |  |  |  |  |  |  |  |  |
| **Team Members** |  |  |  |  |  |  |  |  |  |

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|  | **10. Graph the results for both your individual data and class average in your lab under the data section**. For this graph you will need to determine the following:  a. The independent variable \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.  Use this to label the horizontal (X) axis.  b. The dependent variable \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Use this to label the vertical (Y) axis.  Make sure that you TITLE your graph. |
| **Analysis of Results** | 1. Explain the relationship between the change in mass and the molarity of sucrose within the dialysis bags.  2. Predict what would happen to the mass of each bag in this experiment if all the bags were placed in a 0.4 M sucrose solution instead of distilled water. Explain your response.  3. Why did you calculate the percent change in mass rather than simply using the change in mass?  4. A dialysis bag is filled with distilled water and then placed in a sucrose solution. The bag's initial mass is 20 g, and its final mass is 18 g. Calculate the percent change of mass, showing your calculations.  5. The sucrose solution in the beaker would have been \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ to the distilled water in the bag? (isotonic or hypertonic or hypotonic) |
| **EXERCISE 1C: Water Potential** | In this part of the exercise you will use potato cores placed in different molar concentrations of sucrose in order to determine the water potential of potato cells. First, however, we will explore what is meant by the term "water potential".  Botanists use the term water potential when predicting the movement of water into or out of plant cells. Water potential is abbreviated by the Greek letter psi () and it has two components, a physical pressure component, pressure potential P, and the effects of solutes, solute potential S. |

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| **P + S** |

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|  | **Water will always move from an area or higher water potential**(higher free energy; more water molecules) **to an area of lower water potential**(lower free energy; fewer water molecules). Water potential, then, measures the tendency of water to leave one place in favor of another place. You can picture the water diffusing "down" a water potential gradient.  Water potential is affected by two physical factors. One factor is the addition of solute which lowers the water potential. The other factor is pressure potential (physical pressure). An increase in pressure raises the water potential. By convention, the water potential of pure water at atmospheric pressure is defined as being zero ( = 0). For instance, it can be calculated that a 0.1 M solution of sucrose at atmospheric pressure (P = 0) has a water potential of -2.3 bars due to the solute (S = -2.3). *A bar is a metric measure of pressure, measured with a barometer, that is about the same as 1 atmosphere. Another measure of pressure is the megapascal (Mpa). 1Mpa = 10 bars.*  Movement of water into and out of a cell is also influenced by the pressure potential (physical pressure) on either side of the cell membrane. Water movement is directly proportional to the pressure on a system. For example, pressing on the plunger of a water-filled syringe causes the water to exit via any opening. In plant cells this physical pressure can be exerted by the cell pressing against the partially elastic cell wall. Pressure potential is usually positive in living cells; in dead xylem elements it is often negative.  It is important for you to be clear about the numerical relationships between water potential and its components, pressure potential and solute potential. The water potential value can be positive, zero or negative. Remember that water will move across a membrane in the direction of the lower water potential. An increase in pressure potential results in a more positive value, and a decrease in pressure potential (tension or pulling) results in a more negative value. In contrast to pressure potential, solute potential is always negative; since pure water has a water potential of zero, any solutes will make the solution have a lower (more negative) water potential. Generally, an increase in solute potential makes the water potential value more negative and an increase in pressure potential makes the water potential more positive. |
| **Procedure** | Work in groups. You will be assigned one or more of the beaker contents listed in Table 1.4. For each of these, do the following:  1. Pour 100 mL of the assigned solution into a labeled 250 mL beaker.  2. Use a cork borer to cut four potato cylinders. Do not include any skin on the cylinders. You need four potato cylinders for EACH beaker.  3. Keep you potato cylinders in a covered beaker until it is your turn to use the balance.  4. Determine the mass of the four cylinders together and record the mass in Table 1.4. Put the four cylinders into the beaker of sucrose solution.  5. Cover the beaker with plastic wrap to prevent evaporation.  6. Let it stand overnight.  7. Remove the cores from the beakers, blot them gently on a paper towel, and determine their total mass.  8. Record the final mass in Table 1.4 and calculate percentage change as in Exercise 1B. Do this for both your individual results and class average.  9. Graph both your individual data and the class average for the percentage change in mass in Table 1.4. |

**Table 1.4: Potato Core: Individual Data**

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| **Contents in Beaker** | **Initial Mass** | **Final Mass** | **Mass Difference** | **Percent Change in Mass** | **Class Average Percent Change in Mass** |
| 1. **Distilled Water** |  |  |  |  |  |
| **b) 0.2 M Sucrose** |  |  |  |  |  |
| **c) 0.4 M Sucrose** |  |  |  |  |  |
| **d) 0.6 M Sucrose** |  |  |  |  |  |
| **e) 0.8 M Sucrose** |  |  |  |  |  |
| **f) 1.0 M Sucrose** |  |  |  |  |  |

**Table 1.5: Potato Core Results: Class Data**

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|  | **Percent Change in Mass of Potato Cores** |  |

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|  | **Group 1** | **Group2** | **Group 3** | **Group 4** | **Group 5** | **Group 6** | **Group 7** | **Total** | **Class Average** |
| **Distilled Water** |  |  |  |  |  |  |  |  |  |
| **0.2 M** |  |  |  |  |  |  |  |  |  |
| **0.4 M** |  |  |  |  |  |  |  |  |  |
| **0.6 M** |  |  |  |  |  |  |  |  |  |
| **0.8 M** |  |  |  |  |  |  |  |  |  |
| **1.0 M** |  |  |  |  |  |  |  |  |  |
| **Team Members** |  |  |  |  |  |  |  |  | **N/A** |

**Graph 1.2: Percent Change in Mass of Potato Cores at Different Molarities of Sucrose**

**\*\* Mrs. Pardue will show how this graph should be constructed.**