**Osmosis and Diffusion Lab**

*In addition to close-toed shoes, you are required to wear long pants or skirt (ankle length) to participate in this lab. You will not be allowed in the lab without these minimum requirements.*

The cytoplasm and extracellular environment of the cell are aqueous solutions. They are primarily composed of water (the solvent), and a variety of dissolved solutes, such as sugars, amino acids, and ions. The plasma membrane of the cell is selectively permeable, allowing water to freely pass through, but regulating the movement of solutes.

Water and some dissolved solutes move via passive diffusion through the plasma membrane. Diffusion is a process where molecules move from an area of high concentration to an area of low concentration. How quickly diffusion occurs is dependent on several factors, such as temperature, particle size, and the concentration difference on either side of the membrane. In these experiments we will observe how these factors can affect diffusion rates.

**Diffusion of a Gas Through a Gas**

In this experiment, we will investigate the effect of molecular weight on diffusion. Two gases will be used in this experiment, ammonia (NH₃) and hydrogen chloride (HCl). When these two chemicals react with each other, they produce ammonium chloride (NH₄Cl) which can be seen as a smoky white precipitate.

\[
\text{NH}_3 + \text{HCl} \rightarrow \text{NH}_4\text{Cl}
\]

The molecular weight of ammonia is 17, and that of HCl is 36.5.

Formulate a hypothesis about the impact of molecular weight on the rate of diffusion of these gases.

Predict the results of the experiment based on your hypothesis (formulate an if/then statement).

This experiment is conducted as a demo at the instructor bench, and your instructor may ask for student volunteers. Anyone actively participating in the experiment must wear protective eyewear and gloves!
Caution!!! Do not let either of these solutions contact your skin. The fumes can cause serious irritation to the eyes, so be sure to wear protective eyewear. Fumes can also irritate the respiratory tract, so keep the containers closed when not in direct use. After the experiment is complete, dispose of the cotton balls in the container on the instructor desk.

For this experiment we will use a glass tube, two cotton balls, two pair of forceps, two rubber stoppers, and the solutions mentioned above. Two volunteers should each take a cotton ball in their pair of forceps. Using the medicine droppers, place 5-10 drops of HCl on one cotton ball, and 5-10 drops of NH₃ (or ammonium hydroxide, NH₄OH) on the other. (The cotton balls should be saturated but not dripping.) Insert the cotton balls in opposite ends of the glass tube, and quickly plug each end of the tube with a rubber stopper.

Note the starting time. ______________________________

Watch the glass tube until the smoky precipitate forms. Mark on the tube with a wax pencil where the precipitate first forms. Record the time. __________

When does the precipitate begin to form? ______________________________

Describe the position of the precipitate in the glass tube ______________________________

What do your results indicate about the effect of molecular weight on diffusion rate?
________________________________________________________________________
________________________________________________________________________

Did you support or reject your hypothesis? ______________________________

Diffusion through Agar

As observed in the previous experiment, molecular weight affects the rate of diffusion. In this experiment, we will observe the diffusion of two dye molecules through an agar gel, which is about 98% water. Because of its high water content, the dye molecules will move freely through the gel. In this experiment, we will consider the effect of temperature on diffusion as well.

Work with members of your group to formulate a hypothesis about the rates of diffusion of methylene blue and potassium permanganate through the agar gel. Based on what you observed in the previous experiment, what do you think will happen in this experiment and why?
________________________________________________________________________
Obtain a Petri dish containing agar. It will have two circular holes precut in its surface. On the undersurface of the Petri dish, label one hole with an “M” and the other with a “P”. There should be a china or sharpie marker available for this. Place 2-3 drops of methylene blue in the “M” hole, and potassium permanganate in the other. Be careful not to let the dye solutions overflow onto the surface of the agar. Record the time. _________________________________

A second petri dish will be prepared by your instructor and placed in the refrigerator. Record the time this second Petri dish was placed in the refrigerator. _________________________________

After 45 minutes to 1 hour, measure the distance the dye has diffused from each well. Translate this into a diffusion rate (mm/min). Record your results in the following table.

<table>
<thead>
<tr>
<th></th>
<th>Room Temperature</th>
<th>Refrigerator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What effect does molecular weight have on the diffusion rate? ________________________________

Did you support or reject your hypothesis? ________________________________

Would the dyes diffuse more rapidly through water or the agar gel? Why? ________________________________

What effect does temperature have on the diffusion rate? ________________________________

Look at the data that you have collected in the diffusion experiments. How has the diffusion rate changed as diffusion occurs in a gaseous, aqueous, and solid environment? ________________________________

________________________________________
Would animal tissues be impacted in a similar way? __________________________

________________________________________________________________________

Osmosis

Osmosis is a type of diffusion involving water passing through a selectively permeable membrane. Water will diffuse from a region of high concentration to a region of low concentration. Three terms are used when referring to solutions separated by a selectively permeable membrane. Isotonic refers to a solution that has the same concentration of dissolved particles (and the same concentration of water) as the cytoplasm of a cell. A solution that is hypotonic has a lower concentration of dissolved particles (and a higher concentration of water) than the cytoplasm of a cell. A hypertonic solution has a higher concentration of dissolved particles (and a lower water concentration) than the cytoplasm of a cell. Because of osmosis, cells placed in hypotonic solutions will take on water and swell. Cells placed in hypertonic solutions lose water and shrink. Cells placed in isotonic solutions will remain relatively unchanged as the water moves in and out of the cell at an equal rate.

In this experiment, cells in slices of vegetables will be placed in beakers containing varying concentrations of sugar solutions. The vegetables that will be used are root vegetables that are the part of the plant used to store carbohydrates. The amount of carbohydrate stored in the plant cells will affect water movement across their cell membranes.

Procedure

Obtain six cups numbered 1 through 6. Place the appropriate sugar solution in each of the six cups as depicted in Table 2. Be sure to use only enough solution to cover the vegetable slice (the cup should be no more than 1/3 full).

Record the type of vegetable that your group is using here.________________________

Weigh each slice to the nearest 0.1 gram and record their individual weights in Table 2 on the row labeled 0 min.-initial weight. Simultaneously place each slice into its respective beaker. At 15-minute intervals, remove the slice with forceps onto a paper towel, flip the slice over to remove excess fluid, and weigh it. After recording the weight, place the slice back into the beaker. Repeat this procedure every 15 minutes over a 45 minute time period. After the final weights are recorded, the vegetable slices and the sugar solutions can be discarded.
Table 2. Weight (in g) of vegetable slices during osmosis experiment.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sugar Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>0 min Initial weight</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td></td>
</tr>
</tbody>
</table>

From the data in Table 2, calculate the percent weight change for each of the six slices in each time interval. Use the following formula to determine percent weight change:

\[
\text{Percent weight change} = \frac{\text{next interval weight} - \text{initial weight}}{\text{initial weight}} \times 100
\]

For example, if your vegetable slice had an initial weight of 7.5 g, and after 15 minutes weighed 8.2 grams:

\[
\text{Percent weight change} = \frac{8.2 \text{ grams} - 7.5 \text{ grams}}{7.5 \text{ grams}} \times 100 = 9.3\%
\]

And if your vegetable slice had an initial weight of 7.5 g, but after 30 minutes weighed 5.9 grams:

\[
\text{Percent weight change} = \frac{5.9 \text{ grams} - 7.5 \text{ grams}}{7.5 \text{ grams}} \times 100 = -21.3\%
\]

Calculate weight changes for each slice during each time interval (you should have 18 calculations). Record them in the table below.

Table 3. Percent weight change in vegetable slices during osmosis experiment.

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Sugar Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>0-15 min</td>
<td></td>
</tr>
<tr>
<td>15-30 min</td>
<td></td>
</tr>
<tr>
<td>30-45 min</td>
<td></td>
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</tbody>
</table>
Generate a graph of your data using Microsoft Excel. Open Excel and in the first column, indicate the time intervals used (0, 15, 30, 45). In the following columns, indicate the percent weight change for each solution, starting with the 0% sugar solution (the row at time 0 should be filled with 0’s).

After your data is entered, highlight the data set (only highlight the data, not column headings if present). Select "Insert", "Scatter" chart. Select the scatter chart that reads "scatter with straight lines and markers”. In the "Chart Tools - Design" tab, select "switch row/column”.

Add the vertical and horizontal axis labels and chart title using the "Chart Tools - Design" tab and the “Add Chart Element” dropdown menu. Add a chart legend using the “Add Chart Element” dropdown menu. To change the legend titles, select the "Select Data" tab under the “Chart Tools – Design” menu. In the Legend Series box, click on "Series 1" and select "Edit". You can now change this label to "0%". Repeat with the rest of the legend. You should now have a printable graph indicating the trends observed by each vegetable slice in each solution.

From your data, which solution(s) was/were hypotonic to the cells in the vegetable?
____________________________________________

Which solutions were hypertonic? _________________________________

Were any isotonic, or nearly isotonic? _________________________________

Now compare your data to the data obtained in other lab groups working with a different vegetable. Were the same solutions hypotonic to their cells? _________________________________

Were the same solutions hypertonic? _________________________________

Were the same solutions isotonic? _________________________________

What does this tell you about solute concentrations in these other vegetables as compared to yours? _________________________________
____________________________________________