Objectives:

1. Review the concept of heat and the processes of diffusion and osmosis. Learn the factors that affect the rate of diffusion.
2. Observe Brownian motion and relate this observation to the concept of heat energy.
3. Measure the speed of diffusion of dyes in a gel, and the speed of diffusion of gases in air. Relate the speed of diffusion to molecular mass and medium.
4. Investigate the process of osmosis across semi- permeable membranes using a physical model.

Introduction

At any temperature above absolute zero, the individual atoms and molecules that constitute any substance are in constant motion. **Heat** is the kinetic energy associated with these random movements. **Temperature** is a measure of the average kinetic energy per particle. Kinetic energy (E_K) depends on both the mass (M) and the velocity (V) of the particle:

$$E_K = \frac{1}{2}MV^2$$

To have the same E_K a particle with larger M must have smaller V. Therefore, at the same temperature, heavier particles move more slowly.

In a solid substance, the molecules rotate and vibrate. When the heat energy becomes large enough, the bonds holding the solid together break, and the substance becomes either liquid or gas. In the liquid or gas phase, the molecules can move more freely in space, because they are no longer bound to their nearest neighbors. If you could follow the movement of any one molecule in a liquid or gas, it would move randomly about as it bounced off of other molecules.

Molecules are too small to see directly, but the heat motion of small particles in water is visible under magnification. This random motion is called **Brownian movement** and was described mathematically by Albert Einstein in 1926.

Diffusion is a net movement of particles in a gas, liquid or solid that results from the random movements of the individual particles. Each particle moves randomly. However, if the particles are more concentrated in one region than in another, there will be a net movement away from the region of high concentration. This net movement occurs because there are more particles available to move out of the region of high concentration than there are to move back in. The diffusion continues until there is no more concentration gradient. (A "gradient" is change over distance).

Several properties of diffusion are important to remember: First, there are three factors that affect how fast the individual particles move. As explained above, heavier molecules move more slowly. Therefore, they diffuse more slowly. **The speed of diffusion is proportional to the inverse of the square root of the molecular mass ($1/\sqrt{M}$).** Second, the speed of diffusing particles also depends on the medium in which diffusion is occurring. For example, diffusion of molecules in a gas is much faster than diffusion in a liquid or solid. In a gas, the molecules are spread out and they travel further between collisions. The third factor is temperature. Particles diffuse more quickly at higher temperatures.

The factors listed above all affect the **rate of diffusion-** how many molecules can diffuse through a path per unit time (moles/second). Other limiting factors are the concentration difference, and the length and the area of the diffusion path. The rate of diffusion is inversely proportional to length of the path, and it is directly proportional to area of the path and the concentration difference between the start and the end of the path. That is, if you double the length of the path you halve the rate, and so on.

Diffusion is an important mechanism for moving molecules over short distances within and between cells. Diffusion is especially important for moving O_2, CO_2, and H_2O. The size of cells and the design of multicellular organisms is constrained by the need for adequate diffusion of these substances. Recall that the S/V (surface/ volume) ratio decreases with increasing size. Surface area limits the rate of diffusion, while the volume of living material determines the need for diffusion. The small size of cells provides a large S/V ratio so that diffusion of O_2 and CO_2 will be adequate. On the other hand, a large multicellular organism has S/V far too small to obtain oxygen across the skin, and therefore requires a gas exchange organ such as a lung. The lungs provide a very large surface area and a very thin barrier between blood and air to facilitate diffusion.

Diffusion is slow, and it is only useful over short distances and toward lower concentrations. Rapid transport of substances over long distances requires **bulk flow** (also called **convection**), in which the medium itself moves. Examples of convection include the ventilation of the lungs by breathing or the flow of the blood through the cardiovascular system. Bulk flow can also occur in cells– recall the circulation of organelles in *Elodea* and *Paramecium*.

Transport across membranes

Molecular transport across cell membranes is a special case. Small, non-polar molecules such as CO_2 and O_2 generally diffuse easily through the phospholipid bilayer. Larger molecules, ions, and polar compounds do not. Diffusion of these substances requires special proteins that form pores or gates in the membrane and is called **facilitated diffusion**. Other solutes are carried across by **active transport,** with molecular pumps that expend energy to move them against their concentration gradient. These mechanisms will be discussed in lecture. Now let's consider the transport of water across membranes.

Osmosis is the diffusion of water across a semi-permeable membrane from a region of low solute concentration to a region of high solute concentration. A **semi-permeable membrane** is permeable to water but not to the solute. If water moves into a closed system, such as a cell, the hydrostatic pressure or the volume must increase. **Osmotic pressure** is hydrostatic pressure that results from osmosis. **Osmotic concentration** is the total solute concentration in a solution. Each solute particle, regardless of its size or type, exerts a similar effect on osmotic pressure.

Why does osmosis occur? A simplistic way to think about osmosis is that the presence of solute lowers the concentration of the water, either by displacing water or by binding to it. Therefore, water will diffuse toward higher solute concentration because the water concentration is lower there. This is the "solvent diffusion pressure hypothesis" of osmosis. Although the explanation seems logical, the exact mechanism of osmosis has been debated, sometimes vehemently, for well over 100 years. Luckily, we can observe osmosis without knowing exactly why it happens.

Cells are contain water and live in water. Their membranes are selectively permeable and the concentrations of solutes inside and outside can differ and can change. Therefore, osmosis and osmotic pressure are very important to in many biological contexts. For example:

1) In medical practice, it is common to transfuse solutions into the bloodstream. These transfusion fluids must have an os-

motic concentration that is similar to that of the intracellular fluid (i.e., they must be **isotonic**). This matching of osmotic concentration inside and outside the cells is necessary because, unlike plant cells, blood cells have no cell wall to resist volume changes due to osmosis. If the solution is **hypertonic**, the cells will shrink, and if it is **hypotonic** they will expand or even burst.

2) Osmosis of water into plant cells pressurizes them (**turgor pressure**) and provides a kind of **hydrostatic skeleton** that supports the leaves and smaller stems. Loss of turgor pressure leads to wilting. Is the fluid outside the cells normally isotonic, hypertonic, or hypotonic, relative to the intracellular fluid?

Procedures

1- Brownian motion.

Your lab instructor will show a brief video of a physical model of Brownian motion, and then project a highly magnified view of small particles of dye using the teaching microscope. Can you see Brownian motion among the particles? How does the movement differ among particles of different sizes? How big are the smallest particles that have perceptible motion?

2- Diffusion of dyes in agar.

Your instructor will provide Petri plates containing a layer of **agar**. Agar is a polysaccharide, derived from red algae, that forms a stiff gel when reconstituted with water. Several hours ago, a small hole was cut in the agar was filled with a saturated solution of either potassium permanganate (orange) or malachite green (dark green). These compounds differ in molecular mass:

Mass of K-permanganate = 158 g/mole
Mass of malachite green = 356 g/mole

Use a ruler to measure the distance that the two compounds diffused to the nearest mm. Measure from the edge of the hole containing the dye, not the center:

Maximum distance diffused (mm):

K-permanganate ____________

Malachite green ____________

Time diffusing (sec) ____________

Diffusion in the gas phase is faster than in a liquid or solid, because the molecules are further apart and travel further between collisions. However, diffusion in open air generally involves more convection than diffusion. For example, a few minutes after opening a bottle of perfume you can smell it throughout a room. However, even in a fairly calm atmosphere, much of the transport over distance is due to bulk movements of air (convection) that can be clearly seen by observing smoke or watching dust particles in a beam of sunlight.

In order to demonstrate diffusion in a gas, we need to confine the gas in a small space in order to minimize convection.

3- Diffusion of chlorine and ammonia gas

Your instructor has set up one or more glass tubes as diffusion vessels. The ends are plugged with glass wool. At time zero, a vial containing ammonium hydroxide (NH_3OH) will be slipped over one end and vial containing hydrogen chloride (HCl, hydrochloric acid) will be placed on the opposite end of the tube. Ammonia gas (NH_3) will diffuse from one end and chlorine gas (Cl_2) will diffuse from the other. Do not touch or breath on the tube during this time.

Where the two gases meet within the tube, ammonium chloride (NH_4Cl) will form a ring of white precipitate on the glass. Note the time at which the precipitate first becomes visible, and mark the position on the glass, and measure the distances that each gas diffused.

Distance diffused (mm):

Cl_2: ________________

NH_3: ________________

Time diffused (s): ___________

Find the molecular masses of these gases by looking up the mass of each component atom in the periodic table of the elements. There is a link to the periodic table on the 121 lab page. Remember to use atomic mass (not atomic number) to add up the masses of each atom in the molecule:

Mass of Cl_2 = _______g/mole

Mass of NH_3 =_______g/mole

4– Osmosis, turgor, and plant cells.

Observe the demonstration of lettuce leaves that have been immersed in solutions of different salt concentration for several hours. Pick the leaves up and compare their consistency. How do you explain the differences?

5– Osmosis in model cells

A. Work in groups of 4.

B. Obtain 4 pieces of wet dialysis tubing, each 15 cm long. With each piece, fold about 1 cm of one end up, and then fold again before closing the end tightly with a clip.

C. Label each bag by inserting a small piece of paper with the letter (A, B, C, or D) written in pencil.

D. Add the following solutions to each bag, exclude the air, then fold and close the other end with a clip.

   Bag A– 10 ml of water
   Bag B– 10 ml of water
   Bag C– 10 ml of 25% sucrose
   Bag D– 10 ml of 50% sucrose

   Each bag should be the same size. Don't fold the ends excessively– leave the bags at least 8 cm long, with plenty of room to expand. Make sure that the folds and clips are done neatly so that the bags will be water-tight.

E. When the bags are filled and closed, rinse each one briefly with water, then carefully blot it on a white paper towel to remove any excess water (the brown towels are not absorbent!). After blotting, weigh each bag to the nearest 0.1 gram. Note the time.

F. Place bag A in a beaker of 50% sucrose solution. Place bags B, C, and D in a bowl of water (0% sucrose).

G. Every 15 minutes for the next hour, remove the bags, blot them to remove excess water, and weigh them. Handle carefully and return them to the proper bath as soon as possible.

H. Record the weights and the changes in weight in the tables on the next page.

Dialysis tubing could save your life
This cellulose tubing is used in machines that cleanse the blood of persons with kidney failure. Blood is routed through the tubing, which is surrounded by an isoosmotic dialysis solution. The tubing has tiny pores and is permeable to small molecules, such as the waste product urea, which diffuse out of the blood into the dialysis fluid. The tubing retains the blood cells and larger molecules such as proteins and sugars. The "clean" blood is then returned to the patient.

Table 1. Weights of dialysis bags vs time.

Time	Weight (g)			
	Bag A	Bag B	Bag C	Bag D
Initial	______	______	______	______
15 min	______	______	______	______
30 min	______	______	______	______
45 min	______	______	______	______
60 min	______	______	______	______

Table 2. Change in relative weight of bags (% of initial mass) vs time.

Time	%Change in Weight (g)			
	Bag A	Bag B	Bag C	Bag D
15 min	______	______	______	______
30 min	______	______	______	______
45 min	______	______	______	______
60 min	______	______	______	______

Assignment- Lab Report

Your lab report should be based upon procedures 2, 3, and 4. Review the general instructions for lab reports presented earlier. Some details for this report follow:

Introduction

The introduction of your lab report should include the general **hypotheses** regarding diffusion and osmosis and the specific **predictions** for each of the experiments. A hypothesis is an assumption or possible description of how the system works- it is more general than the predictions. The predictions are specific forecasts that are based on the hypothesis and the experimental design. You test a hypothesis by testing the predictions. If the predictions are accurate, the results support the hypothesis or are consistent with the hypothesis (but don't "prove" it). The plural of hypothesis is hypotheses.

There are several hypotheses tested by these procedures. In procedures 2 & 3, the hypotheses involve the effects of the particle mass and the medium on speed of diffusion. In procedure 4, one possible hypothesis is that water moves toward a region having higher solute concentration. From that hypothesis, you can make several predictions. For example, you could predict that a dialysis bag containing sucrose solution will gain weight when immersed in pure water.

Methods

State the procedures in your own words. Don't get carried away with details. For example, you don't need to describe how you used a pipette to fill the dialysis bags with the solutions, because that would not affect the results. Try to keep this section (and the others!) clear and brief.

Results

In the text of your results section, describe the results, and make reference to the tables and graph.

Table 1- show the results of the two diffusion rate experiments. Include the names of the diffusing compounds, the medium in

which they diffused, the time they diffused, the distance they traveled, and the speed (distance/time). Be sure to include the units of measurement.

Table 2– results of the dialysis bag experiment. Label columns and rows carefully– don't forget the caption.

Graph 1– Bag mass (as % of initial mass) vs time. Four lines on the graph (one for each treatment). Distinguish the lines by symbol or line type. Label thoroughly. Include a legend.

When graphing in Excel remember– use the "XY (scatter)" option for XY graphs. Do not use the "line" plot option. It looks the same but its not! The "line" option does not give you a true scale on the X-axis. That is, it does not plot a Y value versus a corresponding X value. Rather, it just plots the Y values in a series along the X-axis.

Please turn off the gray backgrounds on the figures (right click- format plot area- area-pattern- none).

Discussion

Compare the results with the predictions. Analyze your results further. For example, theory says that the speed of diffusion of a particle should be proportional to the inverse of the square root of its mass (see introduction). You know the masses of the molecules and you can make specific predictions about their relative velocities. Record the needed information in a table (Table 3):

Table 3– Molecular mass of diffusing compounds. Include the name of compound, molecular mass, $1/\sqrt{M}$, and the speed of diffusion in mm/sec. (This table belongs in the discussion, not the results, because it is not something that you actually measured).

How much faster should NH_3 diffuse than Cl_2? According to the hypothesis, the ratio of their values of $1/\sqrt{M}$ would predict the ratio of their velocities. For example, let's make the prediction for relative rates of diffusion of O_2 gas and H_2 gas:

	M	**$1/\sqrt{M}$**
O_2	32	0.177
H_2	2	0.707

Based upon these figures, you would predict that oxygen gas should diffuse about 1/4 as fast as H_2 gas (because 0.177/0.707=0.25).

Make similar predictions for Cl_2 and NH_3 and compare them with your results. If there is a big discrepancy, can you suggest any possible explanations? Make similar comparisons for the two dyes diffusing in agar. You should also compare the speed of diffusion in agar and gas. The dyes are much heavier than the gases– is the difference in their masses enough to explain the difference in the speed of their diffusion? If not, how does this relate to the fact that the dyes were diffusing in agar, while the gases were diffusing in air?

When discussing the osmosis results– do the data support the predictions? If not, can you suggest why? Is the difference in rate of weight change (the slope of your lines) comparable to the differences in concentration between bags and bath? Is the rate of change (slope) constant over time? If not, what is a possible explanation?

Sources

In addition to your textbook, there are some good links to relevant materials on the 121 lab page. The first two sites are particularly useful. The animations on the "Lab Books" site by Patlak and Watters are well done and will help you to understand the concepts.