Diffusion and Cell Membranes - I

Objectives

1. Define the following terms: *solute, solvent, concentration gradient, osmotic pressure, and selectively permeable.*
2. Define the following processes and identify the characteristics that distinguish them from one another: *diffusion, osmosis, facilitated diffusion*, and *active transport*.
3. Give three factors that affect the rate of diffusion and state whether they increase or decrease the rate.

Introduction

Cells maintain a constant internal environment, a process called homeostasis. In a constant environment enzymes and other cellular components can operate at optimum efficiency. The ability to selectively exchange materials with the environment is one component of the cell's homeostatic mechanism. Ions and molecules, such as sugars, amino acids and nucleotides, must enter the cell, and the waste products of cellular processes must leave the cell. Regardless of the direction of movement, the common mediator of these processes is the cell membrane, or plasma membrane.

The plasma membrane is a fluid, or mobile, mosaic of lipids and proteins. Ions and molecules cross the plasma membrane by a number of processes. Large particles are engulfed by the membrane, forming a vesicle or vacuole that can pass into (endocytosis) or out (exocytosis) of the cell. Some small, electrically neutral molecules diffuse through the spaces between the lipid molecules of the plasma membrane. Others bind to transport proteins embedded in the plasma membrane and transported into or out of the cell.

Atoms, ions and molecules in solution are in constant motion and continuously collide with each other because of their kinetic energy. As the temperature is raised, the speed of movement of the molecules increases and they collide more frequently and with greater force. An observable consequence of this motion is Brownian motion, an erratic, vibratory motion of small particles suspended in water, which is caused by the collisions of water molecules with the particles.

Diffusion also results from the kinetic energy of molecules. If a small crystal of a soluble substance is added to water, molecules of the substance break away from the crystal surface and enter solution. As a consequence of the collisions with water molecules, molecules of the substance move in a random pattern in the solution, but always away from the crystal, with some moving to the farthest reaches of the solution. This process continues until the molecules of the substance are evenly distributed throughout the water (the solvent). In a general sense, in any localized region of high concentration, the movement of molecules is, on average, away from the region of highest concentration and towards the region of lowest concentration. The gradual difference in concentration over the distance between the regions of high and low concentration is called the concentration gradient. The steeper the concentration gradient, the greater the rate of diffusion. The rate of diffusion is also directly proportional to the temperature and inversely proportional to the molecular weight of the diffusing molecules. In other words, the higher the temperature the faster the rate of diffusion, but larger molecules move slower than smaller molecules at the same temperature.

Small, electrically neutral substances diffuse into and out of cells by passing through the spaces between the lipids of the plasma membrane or by dissolving in the lipids or proteins of the membrane. Substances that are large or electrically charged cannot pass through membranes. Membranes that block or inhibit the movement of molecules are called differentially permeable, or selectively permeable. Selective permeability explains the phenomenon of osmosis, the diffusion of water across a membrane under certain conditions.

If two solutions containing different concentrations of a solute are separated by a selectively permeable membrane (permeable to water but not to the solute), water will move from the solution with low solute concentration to the solution of high solute concentration. The water flows in this direction because the solution with low solute concentration has a high water concentration and the solution with high solute concentration has a low water concentration. Thus, the water diffuses from a region of high water concentration to a region of low water concentration.

Many ions and molecules important to cells are taken into cells by specific transport proteins found in cell membranes. Facilitated diffusion occurs when such a protein functions as a binding and entry port for the substrate. In essence, the protein functions as a pipeline for the specific substance. The direction of flow is always from high concentration to low concentration. The gradients are maintained because frequently the molecules are metabolically converted to other types of molecules once they enter the cell.

For many other molecules and ions, favorable diffusion gradients do not exist. For example, sodium ions are present at higher concentrations outside mammalian cells than inside the cells, yet the net movement of sodium ions is from the inside to the outside of the cell. Likewise, potassium ions are found inside mammalian cells at significantly higher concentrations than outside the cell, but the net movement of potassium ions is from the outside to the inside of the cell. For such molecules and ions, cellular energy must be used to transport the molecules across the plasma membrane. Active transport occurs when transport proteins in the cell membrane bind with the substrate and use cellular energy to drive the “pumping” of the molecules into or out of the cell, *against* the concentration gradient.

In today’s lab you will observe Brownian motion, osmosis, and diffusion in the solid, liquid and gaseous state, and investigate the parameters that affect the rate of diffusion of molecules. In next week’s lab, which is a continuation of your investigation of diffusion and the properties of cell membranes, you will model a semi-permeable membrane and investigate the behavior of different types of cells in hypotonic, hypertonic and isotonic solutions.

Observing Brownian Motion

The vibratory movement exhibited by small particles in suspension in a fluid was first observed by the Scottish botanist Robert Brown in 1827. Brown incorrectly concluded that living activity was the cause of this movement, but we now know that Brownian movement results from the collisions between water molecules and small particles (less than 10 micrometers in diameter) suspended in the water.

To illustrate Brownian movement, place a drop of water on a microscope slide. Dip a dissecting needle into India ink and then touch the tip of the needle into the water drop. (India ink consists of small particles of carbon suspended in a fluid.) Add a coverslip and observe the slide with a high-power objective.

Briefly record your impressions of the movement of the particles. If you gently warm the slide over a light bulb, what effect does this have on the movement of the particles? How do you account for any changes in motion you observe after heating the slide?

Osmosis

The rate of water movement in osmosis can be observed with an osmometer (see figure below). A starch solution in the thistle funnel is separated from the water in the beaker by a dialysis membrane that allows water to pass through but is impermeable to starch. (The starch solution has had food coloring added to it so that you can track any movement of the solution in the thistle funnel.) What do you expect will happen over time in this type of setup?

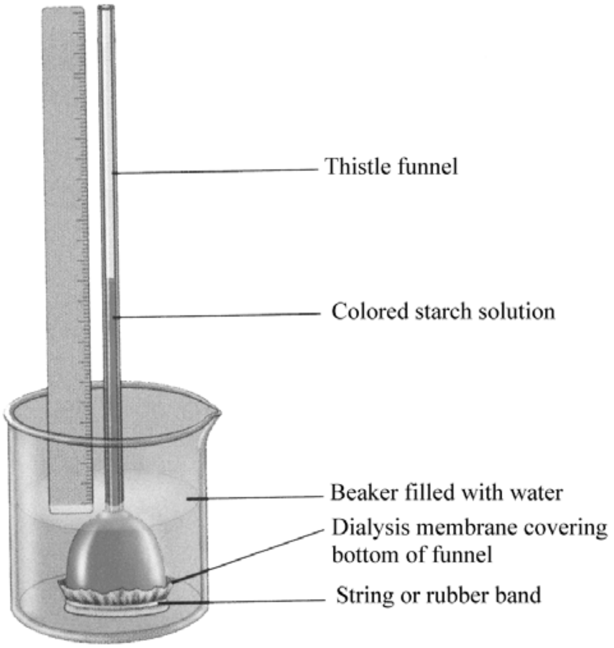

Figure 1. A Simple Osmometer

Early in the lab, measure the height of the column of fluid in the thistle funnel. At intervals of about 20 minutes during the lab, repeat the measurement. Record the time and height of the fluid column in the table below.

Time	Elapsed Time	Height of Fluid Column

Describe what is happening to both the starch and water molecules in the osmometer.

Over time do you expect that the rate of water movement will increase, decrease, or remain the same? Why?

Diffusion in a Solid

The solid we will use is agar, which forms a colloid (a gel-like matrix) when mixed with water, and is clear so you can see into it. Molecules can diffuse through the water-filled channels in the agar matrix. Your instructor may assign you to a group to carryout this experiment. Alternatively, your instructor may do this experiment as a demonstration and provide you with the data at the end of the lab period.

1. Obtain 6 agar plates for your group. Label one pair of plates 4^{o}C, the second pair RT (for room temperature), and the third pair 37^{o}C.

2. Take off the lids of the first pair of plates. Using a toothpick, place a small crystal of potassium permanganate ($KMnO_4$) on the agar surface of one plate, and a similar amount of methyl orange on the agar surface of the second plate. Be careful not to poke a hole in the agar surface. Replace the lid on each plate.
3. Repeat step 2 for the remaining pairs of plates, taking care to use similar sized $KMnO_4$ crystals and methyl orange on each pair of plates as used in step 2.
4. Place the 4^oC plates in a refrigerator, leave the RT plates in a safe spot on your lab bench, and the 37^oC plates in an incubator set for 37^oC. Record the time you start the experiment.
5. After about 1.5 hours, collect each pair of plates and measure the size of the colored ring around each crystal in millimeters. Record the radius of each ring in the appropriate box in table 1 below. Record the time you make the measurements and calculate the time, in minutes, the plates were sitting.

Start time	End time	Length of time (min)

Table 1.

	Radius of ring (mm) 4^oC	Radius of ring (mm) Room temp.	Radius of ring (mm) 37^oC
Potassium permanganate			
Methyl orange			

6. Calculate the rate of diffusion for each molecule at each temperature using the procedure outlined below.
   a. Convert the radius from millimeters the micrometers by multiplying the radius in millimeters by 1000.
   b. Divide the resulting number by the number of minutes the plates were sitting. Record this result in the appropriate box of the following table.

Table 2. Rate of diffusion (μm/min.)

	4^oC	Room temperature	37^oC
Potassium permanganate			
Methyl orange			

Use this data to answer the following questions.
1. The molecules of which substance diffused more rapidly?

2. The molecular weight of $KMnO_4$ is 158 and the molecular weight of methyl orange is 327. What relationship is there between the molecular weight of the substance and the rate of diffusion?

3. What relationship is there between the rate of diffusion and temperature? What reason can you give to explain this relationship?

Diffusion in a Liquid

In this experiment we will be determining the rate of diffusion of $KMnO_4$ in water at room temperature. We will then compare this rate with the rate of diffusion of $KMnO_4$ in agar at the same temperature (from the table above).

1. Place some room temperature water in a glass Petri dish and place the Petri dish over a thin, flat metric ruler.
2. Using tweezers place a crystal of $KMnO_4$ directly over one of the millimeter lines of the ruler and record the time.
3. After 10 minutes, measure the distance the color has moved. Record the final time, length of time and distance moved in the table below.
4. Calculate the rate of diffusion of the $KMnO_4$ using the procedure described above and record it in table 3 below.

Table 3.

	Start time	End time	Length of time (min)	Distance moved (mm)	Diffusion rate (μm/sec)
Water					
Agar					

Transfer the appropriate data from table 2 above and use the data in table 3 to answer the following questions.

1. In which experiment is diffusion the fastest?

2. How can you explain this difference in speed?

Diffusion in a Gas

This experiment is an optional demonstration and will be done at the discretion of your instructor. **It should be noted that the two chemicals involved have a real potential to be harmful and should be treated with extreme caution.** The diagram below illustrates the experimental setup.

1. Remove the rubber stoppers from the end of the glass tube and simultaneously dip one of the cotton tipped applicator sticks into concentrated HCl and the other into concentrated NH_4OH.
2. Simultaneously reinsert the stoppers into the glass tube.

3. Look for the formation of a white ring inside the tube. This is NH_4Cl, a white salt formed when HCl and NH_3 meet.
4. Measure the distance each gas traveled and record the results in table 4 below and use the data to answer the following questions.

Table 4.

	HCl	NH_3
Distance traveled		

1. The molecular weight of HCl is 36 and NH_3 is 17. Which gas did (should) diffuse the fastest?

2. Calculate the following values: the ratio of the distances, the ratio of the molecular weights, and the ratio of the square roots of the molecular weights. Is the rate of diffusion directly or inversely proportional to the molecular weight or the square root of the molecular weight?