Membrane Permeability and Transport

Objective:

To explain in words or diagrams the process of diffusion and the movement of substances across the cell membrane, at the level of 85% proficiency for each student.

In order to achieve this objective, you will need to be able to:

1. Determine the rate of diffusion of substances with different molecular weights.
2. Explain the movement of substances across an artificial membrane.

Part 1

Materials:

Group Supplies

Diffusion of Dyes with different molar masses through an Agar Gel

10 microliter pipettes
petri dish containing agar gel
millimeter ruler
methylene blue (concentrated solution)
potassium permanganate (concentrated solution)

Diffusion of solutes and solvents through nonliving membranes

Three dialysis sacs*
small funnel
10 mL graduated cylinder
wax marker
three beakers (250 mL).

40% glucose solution
10% NaCl solution

hot plate
test tube holder
test tube rack
3 test tubes
dropper bottle of Benedict's solution
silver nitrate solution

Diffusion of Dyes with different molar masses through an Agar Gel

Methods:

1. ***Avoid contact between your skin and the dye.*** Inject 10 microliters of potassium permanganate dye into the agar gel about **1 cm from one edge** of the agar plate. Inject 10 microliters of methylene blue crystals into the agar about **1 cm from the opposite edge** of the agar plate. Use a millimeter ruler to measure the *radius* of each of the spots of dye. Record these values for zero (0) time.
2. At 15-minute intervals, measure the *radius* of each of the spots dye. These observations should be continued for 1 1/2 hours, and the results recorded in the chart below.
3. Compute the rate of the dye diffusion through the gel. The rate is the change in radius (Δ mm) divided by the change in time (Δ min).
4. ***After completion, place the agar plates in the regular trash can***.

Results:

Time (min)	Diffusion of methylene blue (MW = 320)		Diffusion of potassium permanganate (MW = 158)	
	Radius of Dye spot (mm)	Rate of Diffusion Δ mm/Δ min	Radius of Dye spot (mm)	Rate of Diffusion Δ mm/Δ min
0				
15				
30				
45				
60				
75				
90				

Diffusion of solutes and solvents through nonliving membranes:

Methods:

1. Number three beakers 1 to 3 with the wax marker, and fill beakers #1 and 3, each with **100 mL** of distilled water, and fill beaker # 2 with **100 mL** of 40% glucose solution.
2. Prepare the three dialysis sacs* one at a time. Fold over one end of the sac and tie it securely with string. Open the other end by wetting and rubbing between your thumb and index finger. Using a funnel, place **10 mL** of the specified liquid in each (***see Table 1 below***). Press out the air, fold over the open end of the sac, and tie it securely. Before proceeding to the next sac, quickly and carefully blot the sac dry by rolling it on a paper towel, and weigh it. Record the weight in Table 1.
3. Drop each sac into the beaker with the same number (***see Table 1 below***). Be sure the sac is completely covered by the solution in the beaker (add more solution if necessary). Keep the sacs undisturbed in the beakers for **1 hour**. (Use this time to continue with other experiments).
4. After an hour, quickly and gently blot all three sacs dry. Weigh each sac and record their weights in Table 1.
5. Make sure a beaker of water on the hot plate is boiling.
6. ***Measure glucose for Sac#1 and Beaker #1***: Place **5 mL** of Benedict's solution in each of two test tubes. Put **4 mL** of the beaker fluid into one test tube and **4 mL** of the sac fluid into the other. Mark the tubes for identification and then place them in a beaker containing **boiling water** for 2 minutes. Remove from the boiling place in a test tube rack to cool. If a green, yellow, or rusty red precipitate forms, the test indicates the presence of glucose. Green = low glucose, yellow = moderate glucose, and rusty red = high glucose. If the solution remains the original blue color, the test indicates no or very little glucose. Record the reactions to the Benedict's solution in Table 2.
7. ***Measure sodium chloride for Beaker #3***: Take a **5mL** sample of the beaker fluid and put it in a clean test tube. Add a drop of silver nitrate. The appearance of a white precipitate or cloudiness indicates the presence of AgCl (silver chloride), and thus the presence of sodium chloride. Record the reaction to the silver nitrate in Table 2.
8. Make sure you recorded the reactions to the Benedict's solution and silver nitrate in Table 2.
9. ***After completion, wash all glassware and test tubes, in soapy water, rinse, and dry. Return glassware upside down on the counter, and test tubes upside down in the test tube racks. Place the dialysis tubing into the normal trash can***.

*Dialysis sacs are selectively permeable membranes with pores of a particular size. Although living membranes depends on more than just pore size, dialysis sacs will allow us to examine permeability due to this factor.
The instructor will demonstrate how to prepare the dialysis sacs for use.

Results:

Table 1. Weight of Sacs before and 1 hr after Submerging

Sac / Beaker solution	Weight of Sac Before submerging	Weight of Sac 1 hr After submerging
Sac 1: 10 mL 40% glucose Beaker 1: 100mL distilled water		
Sac 2: 10 mL 40% glucose Beaker 2: 100 mL 40% glucose		
Sac 3: 10 mL 10% NaCl Beaker 3: 100 mL distilled water		

Table 2. Reaction to Benedicts Solution and Silver Nitrate

Sac / Beaker solution 1 hr After submerging	Benedicts: 5 mL Glucose high, medium or low	Silver Nitrate: 1 drop NaCl yes or no
Sac #1: 40% glucose; 4 mL sample		XXX
Beaker #1: distilled water 4 mL sample		XXX
Beaker #3: distilled water 5 mL sample	XXX	

Discussion:

Diffusion of Dyes with different molar masses through an Agar Gel

1. Why did the dye molecules move?
2. Which dye diffused more rapidly?
3. What is the relationship between molecular weight and rate of molecular movement (diffusion)?

Diffusion of solutes and solvents through nonliving membranes

4. In which of the test situations did net water movement (osmosis) occur?
5. In which of the test situations did net solute movement (dialysis) occur?
6. With what cell structure can the dialysis sac be compared?