Diana Valencia

Laboratory 2- Molecular Activity & Membrane Transport

Purpose: The propose of this lab is to teach us the basic properties of passive transport, diffusion, osmosis, and differential permeability. We will also learn how the idea of filtration works, and we will look at the effects of tonicity on cells.

Procedures:

Procedure for: 2-B: Measurement of diffusion through a liquid Procedure

1. Working in groups, fill three Petri dishes with 40 ml. of 25°C water
2. Drop one crystal of potassium permanganate into each dish. Be sure to use the same amount of potassium permanganate for each dish. Record the time.
3. Measure, in millimeters, and record the largest diameter of the colored spot after 5 minutes.
4. Repeat steps 1-3 for water at 5°C and at 45°C.
5. Construct a graph of ranges and means for each temperature.
6. Based on your knowledge of diffusion, what is an explanation for these results.

Procedure for :2-C: Measurement of diffusion through agar

1. Petri dishes have been filled with agar. Two holes have been made in the agar. Into one hole, place two drops of methylene blue. Into the other hole, place two drops of potassium permanganate. Record the time and immediate diameter of each spot. This will be your time zero measurement.
2. Measure the diameter of each spot in millimeters once every minute for fifteen minutes.Calculate the averages from the data collected by all groups doing this exercise. Summarize these data.
3. Construct a graph of average diffusion diameter versus time for both chemicals.
4. Determine the diffusion rate of each chemical. Which has the fastest diffusion rate, methylene blue or potassium permanganate? Record these results
5. Look up the molecular formula and structure of methylene blue and potassium permanganate in a MerckIndex. Make note of this information.
6. Interpret your result with respect to the information obtained from the MerckIndex.

Procedure for: 2-D: Demonstration of filtration

1. Fold three filter papers into cones and insert them into three separate glass funnels. Wet the papers to make them stick to the glass.
2. Prepare three 100-milliliter solutions of charcoal and water. Make one thick, one medium thickness, and one thin. Record the mass of the charcoal used in each preparation. NOTE: if your “thin” solution continually runs through the filter, making it impossible to count drops, it is too thin; you will need to make all your solutions proportionally thicker
3. Pour 50 ml of each solution, one at a time, into a funnel.
4. Immediately count the number of drops produced per minute. NOTE: it may be easier to count the drops for 15 seconds then multiply by four to obtain drops per minute.
5. Count the number of drops per minute when the funnel is half-filled.
6. Count the number of drops per minute when the funnel is nearly empty.
7. Did the charcoal pass into the filtrate? Which solution had the fastest rate of filtration? What is the driving force behind filtration? What other factors influence the rate of filtration? Do your results illustrate these influencing factors?
8. Repeat these procedures with the remaining 50 ml. of solution.

Procedure: 2-F: Measurement of osmosis

1. Attach dialysis bags filled as much as possible with sucrose solutions securely to the bottom of two open, thin glass tubes. One bag should be filled with a 25% sucrose solution and the other should be filled with a 50% sucrose solution. Make sure ends of the tubes are immersed in the solutions. NOTE: reliable results depend on your ability to tightly seal the dialysis bags.
2. Insert both bags into separate beakers of distilled water making sure the dialysis bags are fully submersed but not touching the bottom of the beakers, and suspend each by gently applying a ring stand clamp to the glass tubes. Check for solution leaking out of the bags.
3. Allow five minutes for the systems to equilibrate.Then, mark the fluid levels of each glass tube with a felt pen. Record the time.
4. Record the fluid level of the glass tubes in millimeters every 10 minutes for 50 minutes.
5. If the fluid level rises to the top of the glass tube sooner than 50 minutes, record the time it took to get there, measure the length in millimeters from the equilibration line to the top of glass tube. Divide that length by the number of minutes to get your rate in mm/min.

Procedure for: 2-G: Measurement of differential permeability of sugar and starch:

*NOTE: In this experiment, chemical indicators will be used to determine the presence of starch and sugar. Lugol's solution, an amber iodine-containing reagent, will turn dark navy blue in the presence of starch. Benedict's solution, a blue cupric (Cu^{+2})solution, when heated in the presence of a reducing sugar, will be reduced to form a reddish precipitate of cuprous oxide (Cu_2O). The Benedict's solution will change different colors, ranging from green to red, depending upon the amount of sugar present.

1. Fill a dialysis bag with a 1% starch – 10% glucose solution. Reliable results depend on your ability to tightly seal the dialysis bag.
2. Tie the bag to a glass rod and suspend it in a beaker of distilled water.NOTE: Test the water from the bottom of the beaker to ensure that it is free of starch and/or sugar.
3. After 15 minutes has passed check the water again for starch and sugar in the following way:
   - Test for starch:
     A. Add 10 drops of Lugol's solution to 5 ml of water obtained from the beaker.
        Reddish color = No starch
        Navy blue color = Starch present
   - Test for sugar:
     A. Add 3 ml of Benedict's solution to 5 ml of water obtained from the beaker. Simmer the solution at a low boil for 5 minutes.
        Blue color = No sugar
        Color change = Sugar present (green = little sugar; yellow =moderate sugar; orange = more sugar; red = lots of sugar)
4. Test the water in the beaker again at 30, 45 and 60 minutes.
5. Record these results. Explain the significance of these findings in relation to the permeability of the dialysis bag.

Procedure for: 2-H: The effects of tonicity on red blood cells-

*DemonstrationTonicity refers to the solute concentration of solutions. Hypertonic solutions have a higher solute concentration than the cells in this solution. Red blood cells should shrink or crenate due to osmotic loss of water in hypertonic solutions. Hypotonic solutions have a lower solute concentration than cells in this solution. Red blood cells in a hypotonic solution swell and will eventually undergo cytolysis due to osmotic gain of water. Isotonic solutions have the same solute concentration as cells in this solution. Red blood cells in isotonic solutions slightly swell and shrink in a dynamic equilibrium with their medium. In this

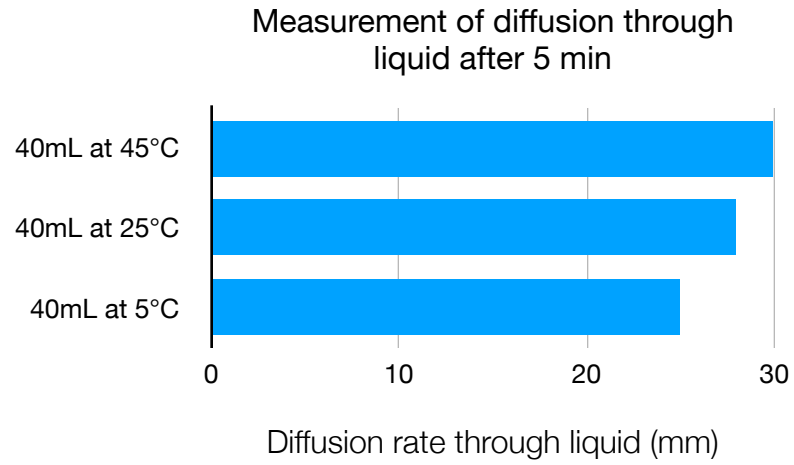
exercise, the effect of hypertonic, isotonic, and hypotonic solutions on red blood cells will be observed.

1. One milliliter of each of the following solutions will be in three separate test tubes.
    a. Distilled water (hypotonic)
    b. Physiological saline–0.85% NaCl (isotonic)
    c. Salt water–2.0% NaCl (hypertonic)
2. A small drop of blood will be added to each tube and the contents thoroughly mixed.
3. A wet mount slide will be made of each solution.
4. Examine each slide under the high-dry lens of a compound microscope.
5. Observe the following:
    a. Hemolysis of cells in the hypotonic solution. (Note the transparent solution.)
    b. Maintenance of cell size in the isotonic solution.
    c. Crenation of cells in the hypertonic solution.
6. Make a drawing of each observation and provide an explanation for each.

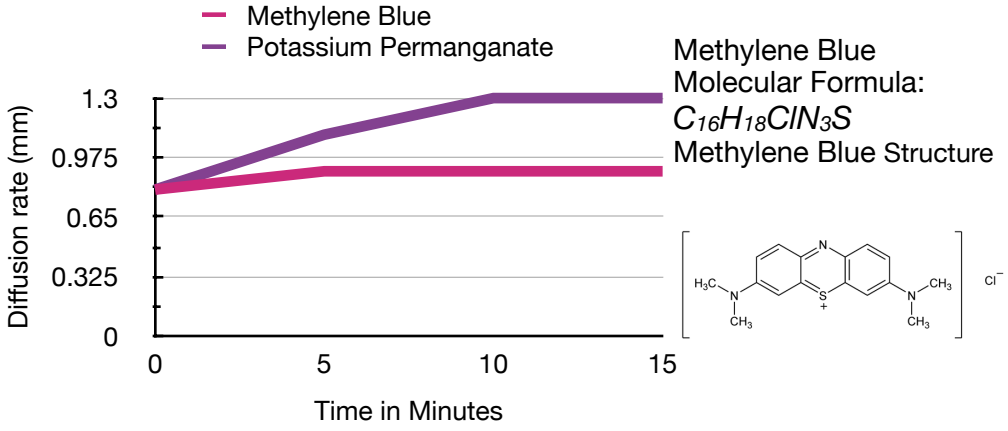
Results:

Results for 2-B: Measurement of diffusion through a liquid Procedure

40mL at 25°C= 28mm 40mL at 45°C=30mm 40mL at 5°C=25

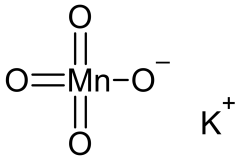


Results for :2-C: Measurement of diffusion through agar



Methylene Blue
Molecular Formula:
$C_{16}H_{18}ClN_3S$
Methylene Blue Structure

potassium permanganate structure



potassium permanganate Formula:
KMnO4

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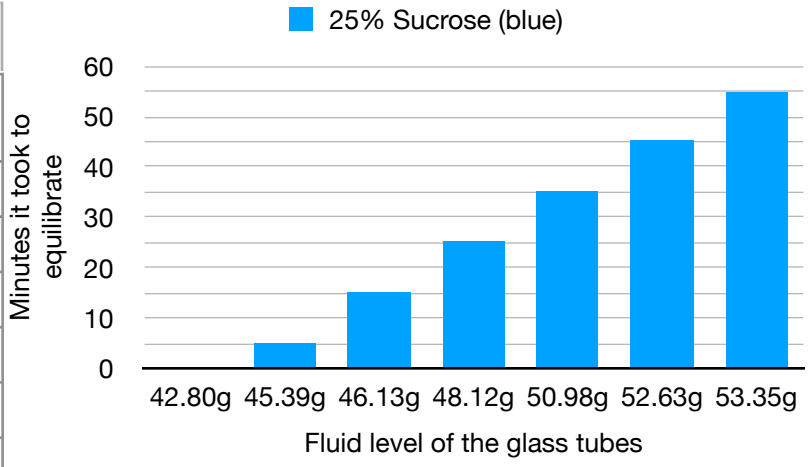
Results: for: 2-D: Demonstration of filtration

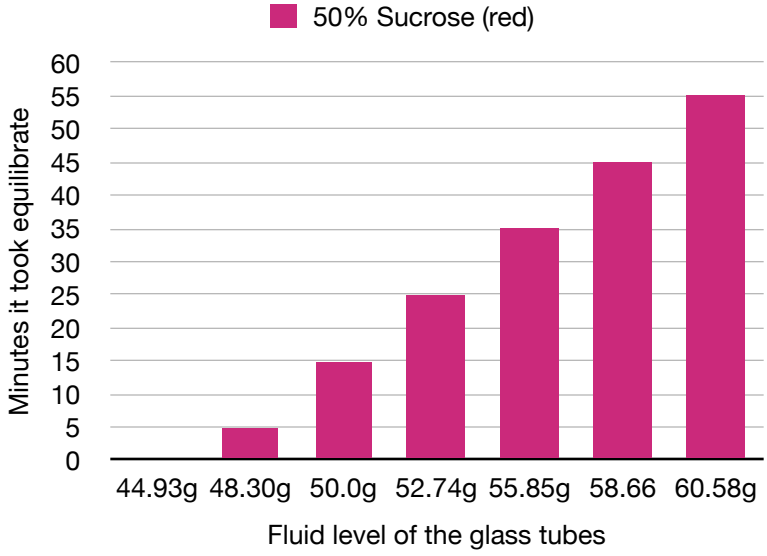
	Thick-7.00g	Medium Thick-1.11g	Thin- 0.39g
Drops per minute	160	156	120
Drops per minute when funnel is half-filled	116	96	80
Drops per minute when funnel is nearly empty	72	56	52

Results for: 2-F: Measurement of osmosis

25% Sucrose (blue)	
Minutes	**Weight in Grams**
0	42.80g
5	45.39g
15	46.13g
25	48.12g
35	50.98g
45	52.63g
55	53.35g



50% Sucrose (red)	
Minutes	**Weight in Grams**
0	44.93g
5	48.30g
15	50.0g
25	52.74g
35	55.85g
45	58.66g
55	60.58g



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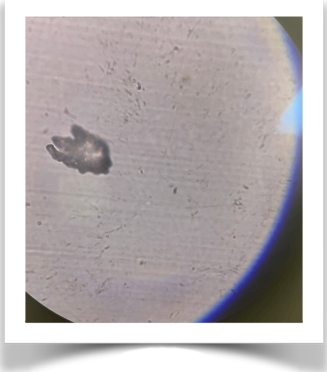
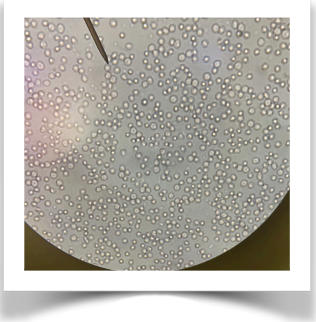
Results for: 2-G: Measurement of differential permeability of sugar and starch:

Minutes	Test for starch	Test for Sugar/ Color
15 Min	No starch in water	Blue=No Sugar
30 Min	No starch in water	Yellow- Moderate sugar
45 Min	No starch in water (Reddish)	Orange=More sugar
60 Min	No starch in water (Reddish)	Reddish= Lots of sugar

Results for: 2-H: The effects of tonicity on red blood cells-

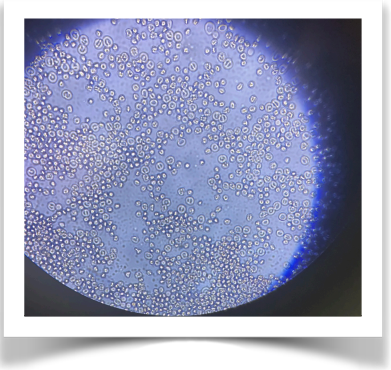
Salt water-2.0% NaCl
Hypertonic





Physiological
Saline-0.85% NaCl
Isotonic

Di Water: Hypotonic
(Swollen)



Discussions:

Discussion for 2-B: Measurement of diffusion through a liquid Procedure
As the temperature increases, so does the rate of diffusion. We noticed that the hotter the temperature was the faster the rate of diffusion was. On the other hand, the colder temperature diffused slowly. We came to a conclusion, that we got these results because as the temperature increases, the energy and the movement of the molecules increase as well, causing them to diffuse through the liquid at a much faster rate.

Discussion for: 2-C: Measurement of diffusion through agar
The results that we got show that methylene blue diffused at a slower rate than the potassium permanganate. This can be because the higher the molecular weight, the slower it will diffuse. A mistake that we may have occurred, is not measuring the size of the diameter correctly, or not measuring it every 15 minutes.

Discussion for: 2-D: Demonstration of filtration
As we were doing this experiment we noticed that the higher the initial mass of the solution was, the faster it filtrated. Another trend it showed is that the amount of fluid left in the funnel also affects the rate of filtration, the more solution in the funnel, the faster the filtration rate and the less solution in the funnel, the slower the filtration rate. An error that we may have made

was not counting every single drop, because there were times when they were going too fast. That may have been that we needed to make it thicker.

Discussion for: 2-F: Measurement of osmosis

We noticed that the 25% Sucrose (blue) had a faster osmotic rate, while the 50% Sucrose (red) had a slower rate. We believe got these results because the higher the weight & sucrose percentage that slower the rate of osmosis was.

Discussion for:: 2-G: Measurement of differential permeability of sugar and starch:

When we began this experiment there was little to no sugar was present in the liquid but as time went on we were able to see an increase of sugar present in the liquid while starch remained undetected the entire time. We concluded that this happened because molecules were too big to pass through the holes present in the dialysis bag while the sugar molecules were small enough to successfully permeate through.

Discussion for: 2-H: The effects of tonicity on red blood cells-:

When a rbc is placed in a hypotonic solution it may swell up and explode, while in a hypertonic solution it will shrink. In isotonic solutions rbcs slightly swell and shrink because it is more balanced.

Conclusion:

In conclusion in this lab we were able to learn the difference between passive and active transport. We learned that hotter temperature diffuse faster than cold temperature. We also learned that weight plays in important role when, it comes to osmosis. In other words, the higher the weight the slower the rate of osmosis is. Permeability is size dependent, smaller molecules can easily pass through a permeable wall while bigger molecules do not. understand the difference between hypertonic, hypotonic, and isotonic. Passive transport doesn't not require energy in order to move.

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