Lab 2- Molecular Activity and Membrane Transport

Purpose: Understand the fundamentals and technicalities of diffusion and membrane transport.

Procedure:

2-B: Measurement of diffusion through a liquid
1. Working in groups, fill three Petri dishes with 40 ml. of 25C 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 5C and at 45C.
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?

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 aMerckIndex.Make note of this information.
6.Interpret your result with respect to the information obtained from theMerckIndex.

2-D: Demonstration of filtration
1. Fold three filter papers into cones and insert them into three separate glass funnels. Wetthe 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? Whatother factors influence the rate of filtration? Do your results illustrate these influencing factors?
8. Repeat these procedures with the remaining 50 ml. of solution.

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.
6.Determine the rate of osmosis for each system. Which system had the fastest osmotic rate, the 25% or 50% sucrose solution? Explain these results.

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 (Cu2O). 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..

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

Tonicity 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

<u>Result:</u>

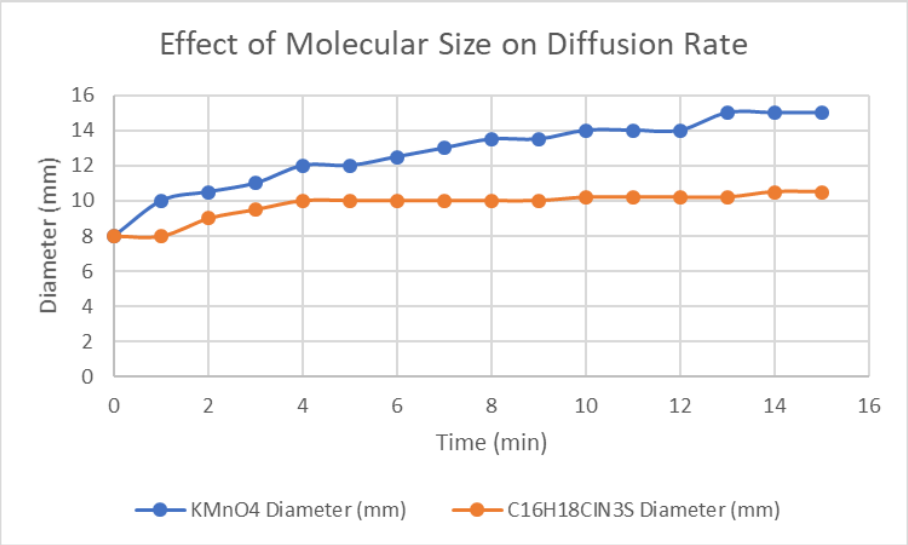
<u>2C:</u>

1. Beginning diameter: 8mm
2.

Minute	$KMnO_4$ Diameter (mm)	$C_{16}H_{18}ClN_3S$ Diameter (mm)
0	8	8
1	10	8
2	10.5	9

3	11	9.5
4	12	10
5	12	10
6	12.5	10
7	13	10
8	13.5	10
9	13.5	10
10	14	10.2
11	14	10.2
12	14	10.2
13	15	10.2
14	15	10.5
15	15	10.5

3.



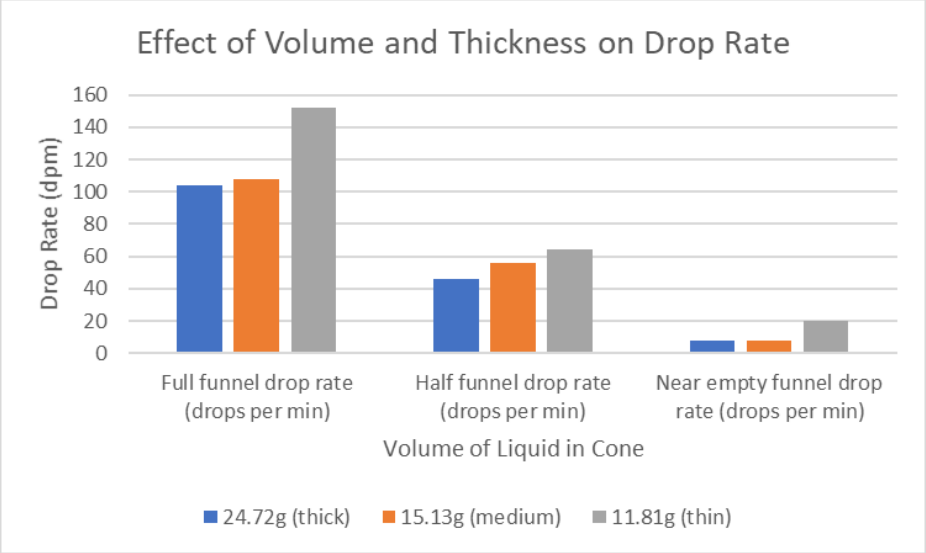
4. Potassium Permanganate: 1mm/min
   Methelyne Blue: .07mm/min

5. Potassium Permanganate: $KMnO_4$
   Methelyne Blue: $C_{16}H_{18}ClN_3S$

2D:

6. For both the thick and thin filters, there was some charcoal in the filtrate. The thin filter had the fastest filtration rate. The driving force behind filtration is gravity. Other factors that affect filtration rate are permeability and viscosity as well as the volume of fluid that is to pass through the filter. The results illustrate these influencing factors in the following table.

Consistency	Full funnel drop rate (drops per min)	Half funnel drop rate (drops per min)	Near empty funnel drop rate (drops per min)
24.72g (thick)	104dpm	46dpm	8dpm
15.13g (medium)	108dpm	56dpm	8dpm
11.81g (thin)	152dpm	64dpm	20dpm

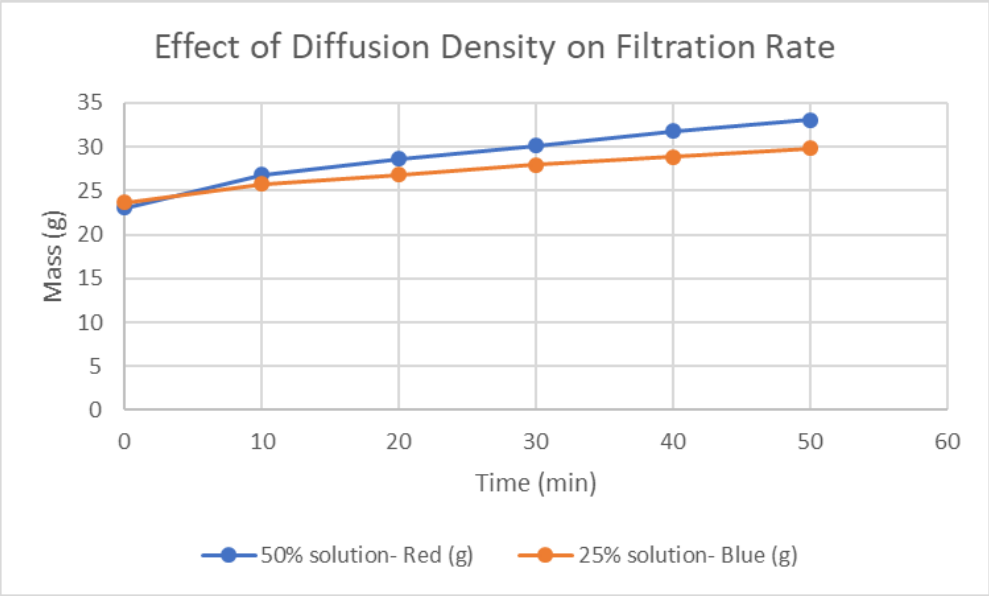


2F:

4)

Time (min)	50% solution- Red (g)	25% solution- Blue (g)
0	23.05	23.65
10	26.84	25.76
20	28.61	26.83
30	30.17	27.93

40	31.79	28.86
50	33.10	29.82



5) Red diffusion rate: .662 g/min
Blue diffusion rate: .5964 g/min
6) The 50% solution has a higher diffusion rate compared to the 25% solution.

2H:

5. DI water (hypotonic solution): the cell has inflated and deformed almost as if it has popped
.85% NaCl (isotonic solution): the cells seem to be in a normal state with no irregularities
2.0% NaCl (hypertonic solution): the cells look denatured, most are deflated

Discussion:

In section 2C there is a possibility for error in the size of the drops ejected from the dropper may affect the results in that if one volume of a drop is bigger or smaller than the other diffusion rate wouldn't be under the same conditions making the experiment less accurate. The measuring method used was also not completely accurate in scientific terms. The measurement was taken from the petri dish with a ruler suspended approximately .5cm from the actual agar, this is not an accurate way of measuring. The ruler should be directly on the item being measured, the space between can be inaccurate due to the angle it is being taken from.
In section 2F, when using the dialysis bags there was some room for small error that may have effect the data collected. These can include the mass of the water left in the strings holding and suspending the dialysis bag affecting mass measurements. The bag not being tied far enough from the top of solution and whether the two are tied and suspended in the same way may affect the osmotic rate due to pressure inside the bag. Lastly for this exercise if the bags have any

unseen leakage due to the method of sealing we used in class can affect the collected rate making it faster than it would be if the bags were properly tied closed.

Conclusion:

Potassium permanganate is a smaller molecule therefore it diffuses faster than Methylene Blue. The thin filter had the fastest filtration rate. The driving force behind filtration is gravity. Other factors that affect filtration rate are permeability and viscosity as well as the volume of fluid that is to pass through the filter. Cells in hypertonic solutions will become deflated due to water diffusing out, isotonic solutions will allow the cells to thrive normally, and hypotonic solutions will cause the cell to increase in size possibly bursting the cell due to water diffusing in.