

## Laboratory 2: Molecular Activity and Membrane Transport

**Purpose:** To investigate the basic properties of passive transport including diffusion, osmosis, and differential permeability. The concept of filtration and the effects of tonicity on cells will also be explored.

### 2B. Measurement of diffusion through a liquid.

1. 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.

### 2C. Measurement of diffusion through agar.

1. Petri dishes have been filled with water. 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 time and immediate diameter of each spot. This will be your time zero measurement.
2. Measure diameter of each spot in millimeters once every min for 15 min.
3. Construct a graph of average diffusion diameter vs time for both chemicals.
4. Determine diffusion rate of each. Which has the fastest diffusion rate, methylene blue or potassium permanganate.
5. Look up the molecular formula and structure of methylene blue and potassium permanganate. Formula for methylene  $C_{16}H_{18}ClN_3S$  molecular weight 319.85  
Molecular formula for potassium permanganate  $KMnO_4$  Molecular weight 158.03

### 2D. Demonstration of filtration.

1. Fold 3 filter papers into cones and insert them into 3 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.
3. Pour 50 ml of each solution, one at a time, into a funnel.
4. Immediately count the number of drops per min produced per minute.
5. Count the number of drops per minute when the funnel is half-filled.
6. Count the number of drops per min when the funnel is nearly empty.
7. Did the charcoal pass into the filtrate? No. Which solution had the fastest rate of filtration? Light. What is the driving force behind filtration? Amount of charcoal. What other factors influence the rate of filtration? How the filter paper is folded. Do your results illustrate these influencing factors? Yes.
8. Repeat these procedures with the remaining 50 ml. of solution.

#### **2F. Measuring 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 50% sucrose solution. Make sure the end of the tubes are immersed in the solutions.
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 gently applying a ring stand clamp to the glass tubes. Check for solution leaking out.
3. Allow 5min 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 10min for 50 min.
5. If the fluid level rises to the top of the glass tube sooner then 50 min, 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. The greater amount of sucrose 50%, has higher solute, this will increase the rate for osmosis.

#### **2G. Measurement of differential permeability of sugar and starch.**

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.

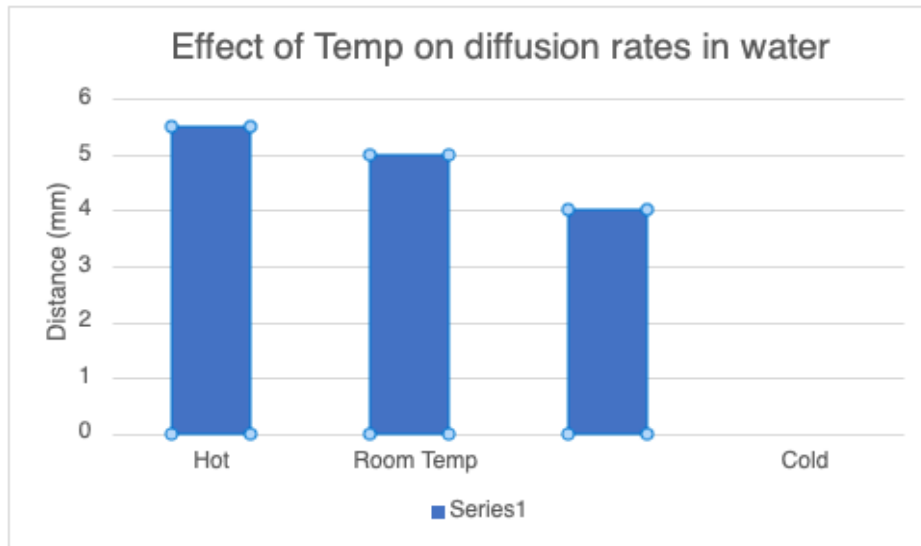
**2H. The effects of tonicity on red blood cells- Demonstration.**

One milliliter of each of the following solutions will be in three separate test tubes.

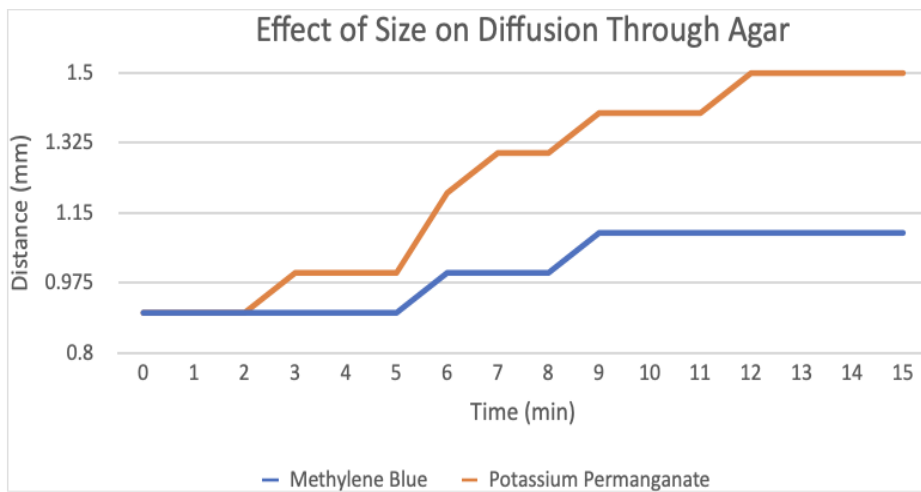
- a. Distilled water (hypotonic)
  - b. Physiological saline – 0.85% NaCl (isotonic)
  - c. Salt water-2.0%NaCL (hypertonic 2.
  - d. A small drop of blood will be added to each tube and the contents thoroughly mixed.
1. A wet mount slide under the high-dry lens of a compound microscope.
  2. Examine each slide under the high-dry lens of a compound microscope.
  3. Observe the following: 1. Hemolysis of cells in the hypotonic solution. 2. Maintenance of cell size in the isotonic solution. 3. Crenation of cells in the hypertonic solution.

**Results:**

**2B.**



**2C.**

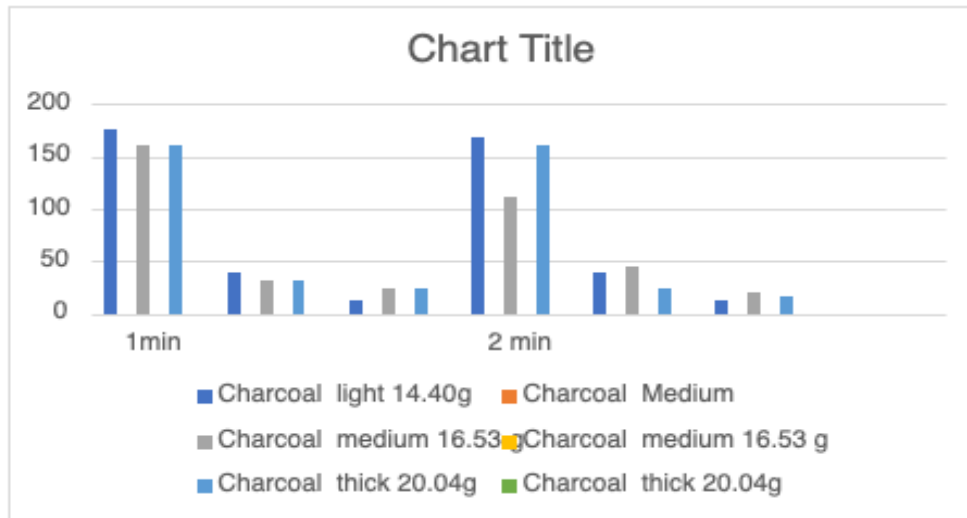


## 2D.

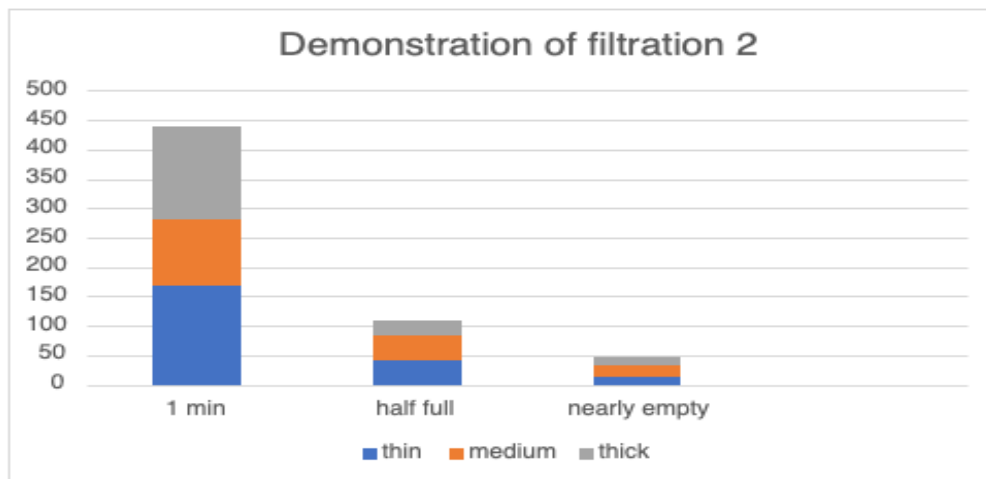
Charcoal

light 14.40g Medium medium 16.53 g thick 20.04g

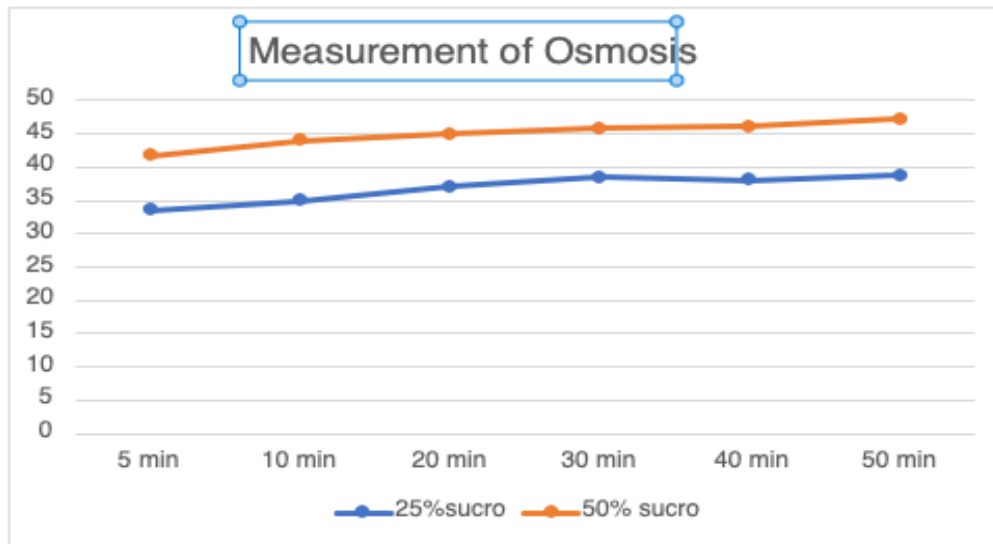
1min	176	160	160
	40	32	32
	12	24	24
2 min	168	112	160
	40	44	24
	12	20	16



## 2E.



2F.



2G. Measurement of differential permeability of sugar and starch.

	15 min	30 min	45 min	60 min
Sugar	0	Moderate	More sugar	More sugar
Starch	0	0	0	0

### Discussion:

With this laboratory we were able to learn more about how osmosis, diffusion and filtrations works.

### Conclusion:

Diffusion refers to the movement of molecules from an area of high concentration. Osmosis is a type of diffusion specifically for water molecules moving across semi-permeable membranes. Filtration is the removal or filtering of the toxins and waste products from the blood by the kidney.