Diffusion across a Semi-Permeable Membrane

SC Academic Standards: 6.L.4A; 6.L.4B; 7.L.3A; H.B.2B; H.B.2C

NGSS DCI: 4-LS1.A; 5-LS1.C; MS-LS1-A; HS-LS1.A

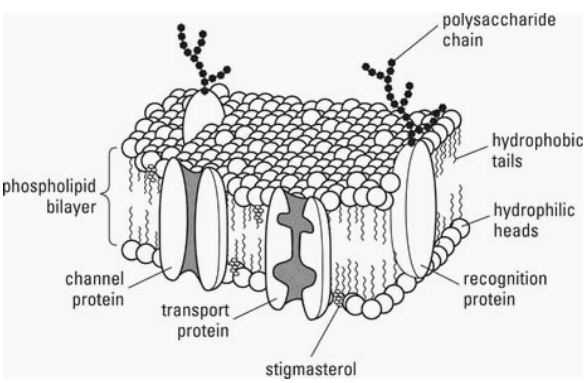
Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.3; S.1A.4; S.1A.5; S.1A.6; S.1A.7

Crosscutting Concepts: Cause and Effect: Mechanism and Explanation; Structure and Function; Scale, Proportion, and Quantity; and Systems Models.

Focus Questions(s): How do molecules get across a cell membrane? What affects the rate of osmosis / diffusion?

Conceptual Understanding: Transport processes which move materials into and out of the cell serve to maintain the homeostasis of the cell.

__Background__: Every cell in your body needs to take in nutrients, oxygen, and raw materials and to export wastes and other substances. But it's not just a random traffic jam! At the boundary of every cell is a cell, or plasma, membrane that regulates what comes in and what goes out of the cell. Plasma membranes consist of a phospholipid bilayer, with each molecule having a hydrophobic tail and a hydrophilic head – so the two lipid strands form a "sandwich with the tails in the middle and the heads facing both inside and outside of the cell. This bilayer is studded with proteins that move laterally within the lipid membrane (like ice cubes floating in a a bucket of water), and is called a "fluid mosaic". Some proteins, and some phospholipids, may also have small chains of carbohydrate attached (these generally function in cell recognition – how a cell tells if another cell is foreign, or "self"). Nutrients, respiratory gases, wastes and inorganic ions must all pass through a plasma membrane on their way into or out of a cell.



*stigmasterol in plants, cholesterol in animals

http://www.cliffsnotes.com/sciences/biology/plant-biology/energy-and-plant-metabolism/membrane-structure

Plasma membranes are **selectively permeable**: some substances can easily enter and exit the cell (or be transported in/out of cells) and others cannot pass without assistance from the embedded proteins. In general, small molecules will **diffuse** *down* a concentration **gradient** (move from the side of the membrane with a high of a particular molecule to the side of the membrane with a lower concentration).

Molecules often have a **net direction** of diffusion until the concentration of that molecule is equal on both sides of a membrane - at which point, the molecules are still diffusing, but there is no net *directional* movement.

When **comparing** two solutions (such as on either side of a plasma membrane), the terms **isotonic**, **hypotonic**, and **hypertonic** are used. Isotonic solutions have the same concentration of a molecule, but a hypotonic solution has less of a molecule than a hypertonic solution, which has more. In general, if a molecule can diffuse, the molecule will diffuse through the membrane from a hypertonic solution <u>into</u> the hypotonic solution. But, if the molecule in question <u>can't</u> diffuse (it is too big, or the membrane lacks the transport proteins), then osmosis will occur instead. **Osmosis** is the diffusion of water across a membrane, and water will always move <u>toward the Hypertonic</u> solution (or from an area of higher water content (and less solute) to an area of lower water content (and more solute) – in effect going down the water gradient. Osmosis of water causes a reduction in the concentration gradient as the solute is diluted. As the gradient decreases, osmosis (diffusion) occurs less quickly.

Substances that are most like phospholipids easily pass through it (this includes non-polar molecules), as do very small molecules like CO_2 gas. Substances unlike the phospholipid membrane (usually polar molecules), and very large molecules, can cross the plasma membrane only with assistance from protein "channels" – these "channels" are not always open (they may be "gated") or may be specific to certain types of molecules (such as a glucose transport protein which only allows glucose through, or a Na+/K+ pump protein which allows Na+ and K+ through, though in opposite directions usually.

Sometimes a larger molecule, or a polar molecule, can't diffuse unless there is a protein "channel" in the membrane that it can move through (and sometimes these channels are blocked, or "gated", which might cause a gradient to be maintained). Facilitated diffusion is when a molecule diffuses (*down* the gradient) through a protein channel. Cells can also *create* concentration gradients by pumping some molecules *up* a concentration gradient, from the side of the membrane with least concentration to the side with a higher concentration of a particular molecule - this is done by **active transport**, also through proteins embedded in the phospholipid bilayer.

Interestingly enough, the disease cystic fibrosis is caused by a defect in the protein that acts as a channel for chloride ions to pass through the plasma membrane of certain cells in the respiratory tract- because of chloride ions aren't transported appropriately, thick plugs of mucus block the airways and infected individuals are prone to bacterial infections here.

The **rate of diffusion** (or osmosis) depends on many things: 1) higher temperature = faster diffusion (temperature is a measure of molecular motion and molecules moving more rapidly register a high temperature); 2) lower molecular weight = faster diffusion; 3) larger concentration difference (gradient) = faster diffusion**. In this lab exercise** you will determine which substances are small enough to pass through the semi-permeable dialysis membrane (our model of a cell membrane) and then you will investigate direction of osmosis and how concentration affects the rate of osmosis. A **model** is a simplified representation of a complex biological structure or process. A model focuses on a few key features in order to help us understand a biological structure. Because a model is simpler than the biological structure it represents, a model does not demonstrate all the features of the actual biological structure.

You will use the model to help **explain** how diffusion occurs across a cell membrane. Our model is a dialysis "bag" or membrane – it is normally used to help a diseased or non-functioning kidney filter blood and remove unwanted wastes (like urea and excess salt) from the blood. Blood goes through the dialysis membrane and wastes diffuse out but the red and white blood cells stay in because they are too large to fit through the dialysis membrane's pores. The dialysis membrane is semi-permeable, but our cells have a selectively permeable plasma membrane (things get through not based on size but on which protein channels the plasma membrane contains, and if the protein channels are "open"). If osmosis (the diffusion of water across a membrane) is occurring, we can measure the directional movement of water into or out of the dialysis tube bag by measuring change in the weight or volume of solution in the bag. If there is no weight gain we can infer that the molecule and/or water did not diffuse across the membrane. Further, we can explore the **rate** of diffusion, which not only depends on the organism's surface area but also temperature, molecular weight of the molecule diffusing, and concentration gradient across the membrane.

Materials:
250 ml beaker or other container (2 per student group)
4 styrofoam cups per student group
1% starch solution, corn or potato (about 10 ml per group)
20% sucrose and 40% sucrose (make a couple of liters of each)
15% glucose solution (about 10 ml per group)
1" dialysis tubing (six 15 cm long pieces per group, kept wet in a plastic bucket/shoebox of water) (Carolina Biological, 1 inch by 50 feet $30)
String (ten 12 cm pieces per group)
Scale, water, paper towels, graduated cylinders, small funnel (or syringe), plastic droppers, and scissors.

(for the **extensions**, you also need iodine, alka-seltzer, carbonated water (flavored water is ok) bromothymol blue, glucose urine test strips)

- To prepare 1% starch solution, mix 10 g of corn starch or potato starch in 50 mL of room temperature distilled water. Bring 1000 mL of distilled water to a full boil. Add the slurry of starch to the boiling water and stir for at least 2 minutes. Starch is insoluble in cold water and needs to be boiled to stay in solution. Allow several hours for the starch solution to cool.
- Prepare 15% glucose solution by dissolving 15 g glucose for every 85 ml of water. Boil, and cool.
- 20% sucrose is made by dissolving 20 g sucrose for every 80 ml water; 40% sucrose is 40 g per every 60 ml water. Boil, and cool.
- Cut the dialysis tubing into 15 cm lengths and soak in distilled water for at least 15 minutes before the activity (dry dialysis tubing gains weight when it is first soaked in water). We suggest that you also precut the 12 cm pieces of string and soak them. Instead of using string, you may provide students with longer pieces of dialysis tubing and have them tie knots in the tubing.

Previous Knowledge: (chemistry): **Diffusion** and **Osmosis** are both passive processes, and molecules move across membranes due to differences in concentration in accordance with chemistry's **Law of Mass Action. Diffusion** results from the *random* motion of molecules which, bumping into each other, spread from areas of high concentration toward areas of lower concentration. The difference in molecular concentration is called a concentration gradient. Diffusion of water across a selectively permeable membrane is called **osmosis**; osmosis results in net movement of water from a solution with a high concentration of free water molecules (low concentration of solutes) to a solution with a low concentration of free water molecules (high concentration of solutes).

Previous Knowledge: (biology): The rate of exchange of substances also depends on the organism's surface area that is in contact with its surroundings. A small organism, like an amoeba, has a large surface area:volume ratio, and so it can accomplish all the exchange it needs by simple diffusion across its body surface. However, a large organism, like a mammal, has a much smaller surface area: volume ratio, so it cannot accomplish all the exchange it needs in this way.

Procedure: *You may wish to do the extensions first, as a demo; the lab second.

Part 1: Investigating diffusion

1. Take two dialysis membranes and either tie a knot in the bottom or double the membrane over and tie it tightly with string. Bags are more easily opened (for insertion of dropper) when wet. Feel free to moisten as needed.
2. Add 4 dropperfuls of 15% glucose to the bag and add 4 dropperfuls of 1% starch to the same bag. Don't tie other end – hold it up and place bag in a beaker of water plus about 10 ml of iodine (Lugols reagent). Let untied top of dialysis bag hang over edge of beaker and use a rubber band to keep it from being submerged.
3. Add 4 dropperfuls of water/bromothymol blue in the second bag. Put dialysis bag in a beaker of carbonated water, let untied top of dialysis bag hang over edge of beaker using a rubber band to keep it from submerging.
4. Watch and Wait for 15-20 minutes. Fill out predictions and expecteations.
5. After 20 minutes, test the first beaker for glucose using a glucose test strip. For both beaker/bag situations, record color changes, if any, that you see.

Substance	**Expected Observations**	
	If this substance crossed the membrane the inside of dialysis bag will look like this:	**If this substance did not cross the membrane the inside of dialysis bag will look like this:**
Iodine (I_3^-)		
Carbon Dioxide (CO_2)		
Bromo. Blue		
Water (H_2O)		
Glucose ($C_6H_{12}O_6$)		
Starch (polysacc. of glucose)		

Table 1. For each substance, indicate how you will know whether it crossed the synthetic membrane. What observation will be different, depending on whether or not this substance crossed the synthetic membrane?

Molecule or Ion (Molecular Formula)	Does it cross the membrane?	
	Prediction	Observation
Iodine (I_3^-)		
Carbon Dioxide (CO_2)		
Bromothymol Blue		
Water (H_2O)		
Glucose ($C_6H_{12}O_6$)		
Starch (polysaccharide made up of many molecules of glucose)		

Table 2. Extension activities: predicting if molecules can diffuse across a dialysis membrane

Part 2: Investigating direction and rate of osmosis. In this experiment, you will set up four separate containers, record data over a 40 minute time span, and construct a graph with 4 data sets (lines).

1. Take 4 dialysis tubes from the bucket. Tie one end of each tube like membrane tightly (either in a knot, or fold over the end and tie with string, cutting string ends short so they don't absorb too much water).

2. Fill 4 styrofoam cups about ¾ full with the following solutions:
   a. Cup 1, 2, 3 = ¾ full of water
   b. Cup 4 = ¾ full with 20% sucrose

3. Fill the bags as follows: (* teachers: these bags / solutions all look alike so maybe assign one student per group one bag, and have them keep track of their bag. The dialysis bags can be set down on the table, perhaps in front of a Styrofoam cup labeled with marker as cup # 1, cup #2 etc.).
   a. Bag 1 – 15 ml of tap water
   b. Bag 2 – 15 ml of 20% sucrose
   c. Bag 3 – 15 ml of 40% sucrose
   d. Bag 4 – 15 ml of tap water
4. Weigh each bag to the nearest tenth of a gram (0.1g) and record your weights on the data table (these are the time zero weights).

5. Once ALL the bags are filled with their solution, tightly tie the other end. At the same time place each bag in its assigned cup and start your timer. Bag #1 (water) will go into cup # 1 (water) and Bag #2 (20% sucrose) will go into cup # 2 (water) and so on.

6. Every 10 minutes for 40 minutes remove each bag and weigh it, then return it to its proper cup.

7. After 40 minutes are up, and your last weight taken, throw cups, dialysis bags, and solutions away.

8. In your data table, calculate a change in weight **(Δ wt)** from the initial weight (time zero) for each bag. On the graph, plot change in weight versus time using all 4 data sets on the same graph (you will need a key).

	Bag 1		**Bag 2**		**Bag 3**		**Bag 4**	
	weight	**Δ wt**	**weight**	**Δ wt**	**weight**	**Δ wt**	**weight**	**Δ wt**
Time 0								
10 min								
20 min								
30 min								
40 min								

Table 3. Change in weight for dialysis membrane bags in a variety of solutions

<u>Data Analysis</u>: Neither starch, sucrose, nor Bromothymol Blue will pass through the dialysis membrane because the molecules are too large to fit through the pores of the semi-permeable dialysis tubing. In contrast, glucose, sucrose, iodine, CO_2, and water molecules are small enough to pass through the membrane.

For the graph, you will want an X axis in the middle of the page, so that you have negative numbers below and positive numbers above. Please note that we are graphing change in weight (*delta weight, or* ***Δ wt***) <u>NOT</u> total weight – so you will need to do some subtraction! And will get negative numbers, especially in Bag #4. You will graph the rates of osmosis for the 4 situations: isotonic water in water, 20% and 40% sucrose in water, and water in 20% sucrose. Bag #1 should not change in weight very much. Bags #2 and #3 should both gain weight, and appear to have swelled. Bag #3 should gain the most weight as the concentration difference between bag and cup was greatest. Bag #4 should lose weight and shrink.

<u>Extensions</u>: This is a good place to talk about **indicator solutions**. You can drop an alka-seltzer into a large beaker of water / bromothymol blue solution – as the CO_2 is released from the alka-seltzer the blue water turns yellow – and you can see it changing first where the CO_2 is released then all throughout the beaker as the CO_2

diffuses. You can also drop food coloring into a tall graduated cylinder, or a tea bag in a glass jar (but the alka-seltzer is fast and dramatic). Then you can introduce the concept of semi-permeable membranes with the dialysis bag - If you put starch in the dialysis bag and immerse the bag in a beaker of water / iodine, the starch, which is too large to fit through the dialysis membrane pores, remains in the membrane bag but the iodine, which is small, diffuses through the membrane and colors the starch blue-black (indicating presence of starch). You can put glucose solution in the cup, and water in the membrane (or vice versa) and similarly use glucose urine test strips to see that glucose is also small enough to fit through the dialysis membrane pores and so diffusion occurs. And, you can put carbonated water in the beaker and a water / bromothymol blue solution in the dialysis membrane bag, and watch the membrane bag turn to green then yellow as CO_2 gas diffuses into the membrane bag. **Starting** with these extensions is often a good way to begin this lesson, and then do the lab – they will be better able to predict which molecules will diffuse and in which direction diffusion will occur.

You may also want to use the first two pages of the computer simulation "Diffusion, Osmosis, and Dialysis (http://molo.concord.org/database/activities/223.html).

In addition, you may want to introduce your students to the phenomenon of osmosis using "Introduction to Osmosis" which includes an investigation using eggs and discussion questions.
(http://serendip.brynmawr.edu/sci_edu/waldron/#osmosis)

Last, refer to the lab outlined in The American Biology Teacher, Vol. 76(4): 265-269 in the article “A simple inquiry-based lab for teaching osmosis” by John Taylor. This lab uses potatoes instead of agar blocks, and has students carve a potato into a 20 gram piece designed so that it will maximize diffusion - as evidenced by the potato gaining weight when placed in salt water for ten minutes. This write up also includes a section where students guess which bucket contains the saltiest water based on how much weight a potato gains.

Reflection Questions:

- **What types of substances do human beings need to take in / eliminate from their bodies? Do individual cells need to do similar things?** (gases, nutrients (food), wastes – yes)

- **Can you think of examples where water moves across a membrane?** (urinary system! Digestive system! We move a lot of water across membranes, particularly in the large intestine, and also in the nephrons).

- **What roles do diffusion and osmosis play in the transport of materials across a plasma (cell) membrane?** (it is how molecules are transported!)

- **How is our model membrane (dialysis bag) like / unlike a real plasma membrane?** (they both are permeable, though the dialysis bag is semi-permeable (meaning anything smaller than pore size can be transported across the membrane, there is no selectivity or "closing the gate / channel". A real plasma membrane would have protein channels and can permit some molecules to cross but not others (regardless of size). Also a real membrane would be capable of active transport and the dialysis membrane is not).

- **Given the scenarios you set up in the procedure, predict direction of flow for each situation.** (Bag 1 is isotonic, so while water molecues are randomly moving there is no net direction and so there should be very little change in weight – possibly a bit of back and forth due to random movement, or water on the string/knots. Bags 2 and 3 are both hypertonic to the beaker solution so both should gain water (and weight) as osmosis occurs. Bag 3 however should gain more weight, and gain water more rapidly, as the concentration difference between bag and beaker is greater – and a bigger concentration difference means faster diffusion/osmosis. These two bags will swell and become turgid. Bag 4 is hypotonic to the beaker, and so water flows out of the bag and the bag shrinks and loses weight).

- **What is the relationship between the concentration of sucrose and the rate of osmosis?** (the rate of osmosis is faster when the concentration difference between the inside of bag and outside of bag is greatest).

- **Which bag and beaker contained solutions that were isotonic to each other?** (Bag 1). **Which bags were hypertonic to the solution in the cups?** (Bags 2 and 3). **Which bags were hypotonic?** (Bag 4).

- **Why do nurses / doctors transfuse normal saline (0.9% NaCl) into dehydrated patients instead of water? Or 5% saline?** (0.9% is isotonic, and won't cause blood cells/tissues to swell or shrink. 5% saline is hypertonic to our body tissues and blood, and so red blood cells would lose water and shrivel up (crenation). Pure water is hypotonic and so water would flow into our red blood cells causing them to swell and possibly burst (hemolysis)).

<u>Models and Explanations:</u> In this lab we explored the diffusion of molecules across a model cell membrane. Our model was semi-permeable dialysis tubing. **A student who demonstrates understanding** of these concepts can explain that diffusion is the main way nutrients and gasses get transported across a cell membrane, and that if the molecule *can* be transported across the model membrane (if it is small enough to fit through the pores) then the *direction* of diffusion will be *down* the **gradient** (from the side of the membrane that has a higher concentration of that molecule towards the side of the membrane that has a lower concentration of that molecule). And, if the molecule can't be diffused, then water will move instead, by osmosis, down the water gradient, or *into the hypertonic* solution. Further, **this student will understand** that diffusion will occur constantly, but there will be a net direction of diffusion when there is a concentration difference across a membrane, and the net direction remains until the two sides of the membrane are equal in concentration, in which case molecules are still diffusing, but with a random non-directional movement (diffusion doesn't *stop*). **This student will be able to compare** our model (semi-permeable) membrane with a real plasma membrane which is selectively permeable, and will be able to explain the role of transport / channel proteins in facilitated diffusion (and how sometimes these proteins are gated, or closed) and will be able to discuss active transport (ATP required; direction of transport being *up* the gradient). **Last, a proficient student can predict direction of osmosis given a situation and explain how and why** some of our model membranes in cups of solution gained weight, lost weight, or remained the same weight.

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Student Worksheet:

Plasma membranes are **selectively permeable**: some substances can easily enter and exit the cell (or be transported in/out of cells) and others cannot pass without assistance from the embedded proteins. In general, small molecules will **diffuse** *down* a concentration **gradient** (move from the side of the membrane with a high of a particular molecule to the side of the membrane with a lower concentration).

Molecules often have a **net direction** of diffusion until the concentration of that molecule is equal on both sides of a membrane - at which point, the molecules are still diffusing, but there is no net *directional* movement.

When **comparing** two solutions (such as on either side of a plasma membrane), the terms **isotonic**, **hypotonic**, and **hypertonic** are used. Isotonic solutions have the same concentration of a molecule, but a hypotonic solution has less of a molecule than a hypertonic solution, which has more. In general, if a molecule can diffuse, the molecule will diffuse through the membrane from a hypertonic solution <u>into</u> the hypotonic solution. But, if the molecule in question <u>can't</u> diffuse (it is too big, or the membrane lacks the transport proteins), then osmosis will occur instead. **Osmosis** is the diffusion of water across a membrane, and water will always move <u>toward the Hypertonic</u> solution (or from an area of higher water content (and less solute) to an area of lower water content (and more solute) – in effect going down the water gradient. Osmosis of water causes a reduction in the concentration gradient as the solute is diluted.

The **rate of diffusion** (or osmosis) depends on many things: 1) higher temperature = faster diffusion (temperature is a measure of molecular motion and molecules moving more rapidly register a high temperature); 2) lower molecular weight = faster diffusion; 3) larger concentration difference (gradient) = faster diffusion. **In this lab exercise** you will determine which substances are small enough to pass through the pores of the semi-permeable dialysis membrane (our model of a cell membrane) and then you will investigate the direction of osmosis and how concentration affects the rate of osmosis.

- What types of substances do human beings need to take in / eliminate from their bodies? Do individual cells need to do similar things?

- Can you think of examples where water moves across a membrane?

- What roles do diffusion and osmosis play in the transport of materials across a plasma (cell) membrane?

- How is the dialysis membrane like / unlike a real plasma membrane?

Part 1. Diffusion. For each substance, indicate how you will know whether it crossed the synthetic membrane. Will there be a color change (describe it), depending on whether or not this substance crossed the synthetic membrane?

Substance	**Expected Observations**	
	If this substance crossed the membrane the inside of dialysis bag will look like this:	**If this substance did not cross the membrane the inside of dialysis bag will look like this:**
Iodine (I_3^-)		
Carbon Dioxide (CO_2)		
Bromo. Blue		
Water (H_2O)		
Glucose ($C_6H_{12}O_6$)		
Starch (polysacc. of glucose)		

Table 1. Color of dialysis bag contents if the molecule did, or did not, cross the membrane

Molecule or Ion (Molecular Formula)	**Does it cross the membrane?**	
	Prediction	**Observation**
Iodine (I_3^-)		
Carbon Dioxide (CO_2)		
Bromothymol Blue		
Water (H_2O)		
Glucose ($C_6H_{12}O_6$)		
Starch (polysaccharide made up of many molecules of glucose)		

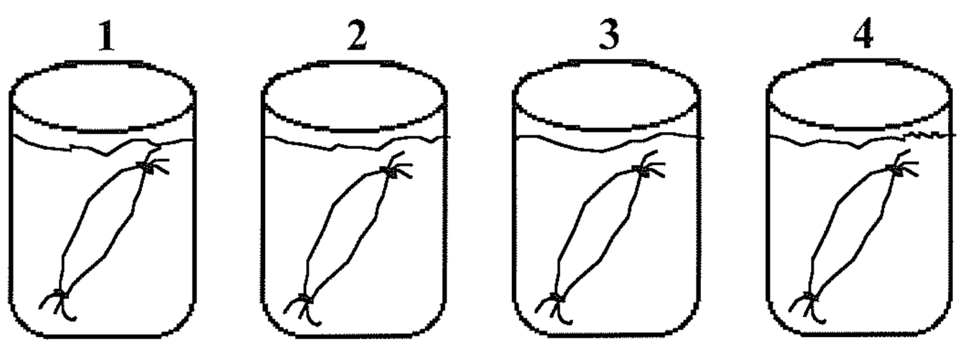
Table 2. Predictions about which molecules will cross the dialysis membrane.

- Based on your results, what characteristic appears to determine which molecules and ions can cross the synthetic semi-permeable membrane (our model of the plasma membrane)?

Part 2. Direction and Rate of Osmosis. In this experiment we will use sucrose, which, as a disaccharide, is too large to fit through the dialysis membrane bag's pores. The dialysis membrane is modeling a plasma membrane, but it is semi-permeable instead of selectively permeable. We will set up 4 difference situation and monitor osmosis over 40 minutes. As osmosis occurs we will take note of direction (bags that gain weight have water moving by osmosis into them; bags that lose weight are losing water) and we can calculate rate of osmosis as change in weight /time.

The 4 situations are shown below. **Hypothesize**: Which bags will gain weight (swell), which will lose weight (shrink) and which will stay the same in weight?

Draw an arrow indicating your hypothesized direction of osmosis for each situation. Then, label each bag as isotonic, hypertonic, or hypotonic as compared to the solution in the cup.



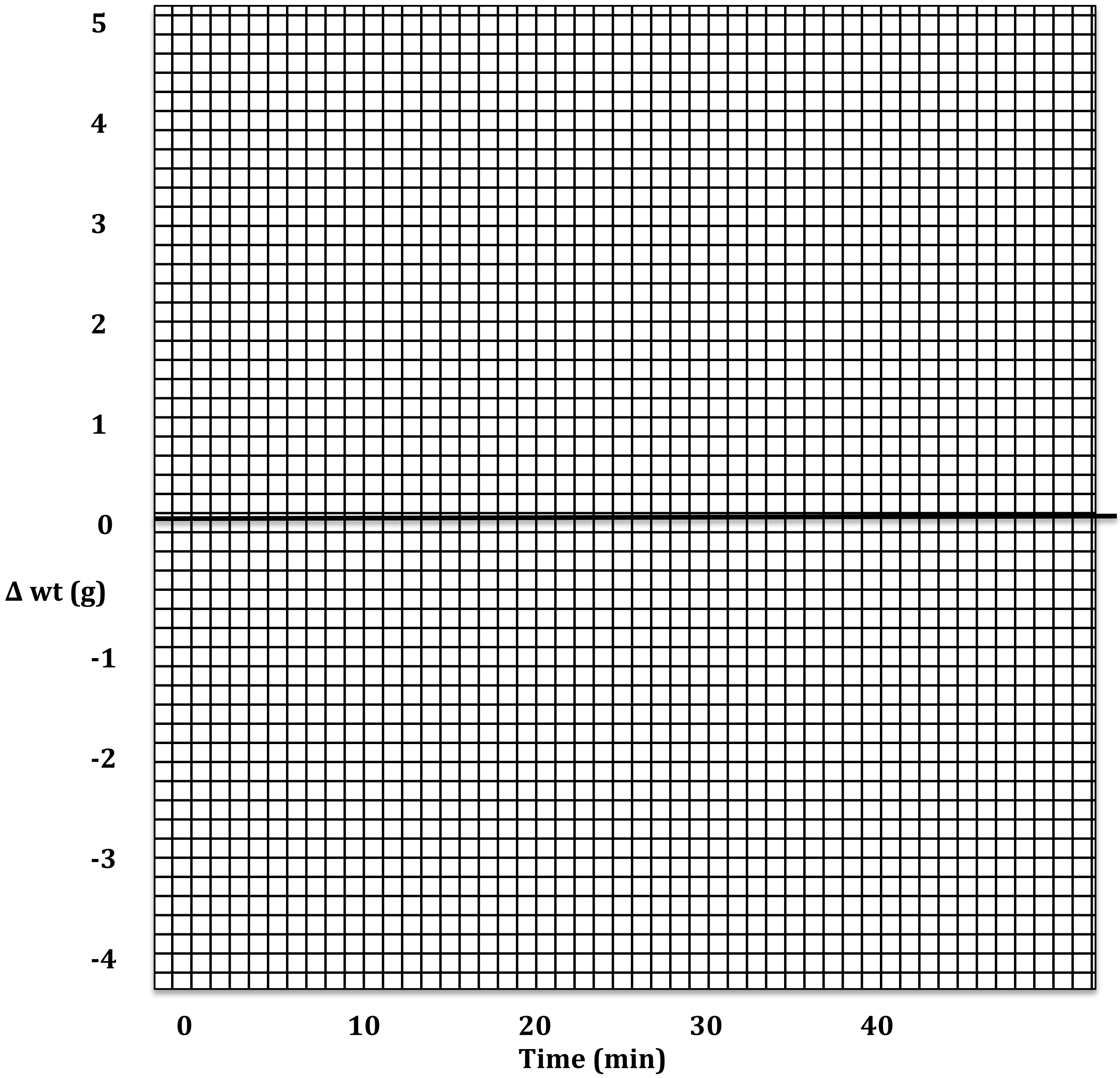
	1	2	3	4
Bag Contents:	Water	20% Sucrose	40% Sucrose	Water
Cup Contents:	Water	Water	Water	20% Sucrose

Tonicity:
Bag 1 is ___________ Bag 2 is ___________ Bag 3 is ___________ Bag 4 is _____________

	Bag 1		**Bag 2**		**Bag 3**		**Bag 4**	
	weight	**Δ wt**	**weight**	**Δ wt**	**weight**	**Δ wt**	**weight**	**Δ wt**
Time 0								
10 min								
20 min								
30 min								
40 min								

Table 3. Change in weight of dialysis membrane bags in a variety of solutions.

Figure 1. ____



- **If rate = rise / run (Δwt/min), calculate the rates of osmosis for each bag.**

Bag 1 = ___________ Bag 2 = ___________ Bag 3 = ____________ Bag 4 = ____________

- **Which bag and beaker contained solutions that were isotonic to each other?**
- **Which bag(s) contained a hypertonic solution?**
- **Which bag(s) contained a hypotonic solution?**

- **What is the relationship between concentration of sucrose and rate of osmosis?**