Teacher's Guide

Linear Diffusion and Cell Signaling

Grade Level: High

Subject area(s): Biology, Chemistry

Time required: (2) 55 minute periods

Learning Objectives: 1. To accurately describe diffusion; 2. To understand importance of cell signaling; 3. To understand concentration and dilution

Summary: In this activity, students explore the concept of diffusion as a component of cell communication. Specifically, students will explore how the rate of diffusion varies with distance and concentration gradient. They will use a pipet with water and dye to observe a macro-model of diffusion. Additionally in the optional extension, students may explore how diffusion is affected by other factors such as medium type, temperature, molecular weight, shape of container (non-linear diffusion) or any other variable.

Purpose. This lab is designed to help students understand:

- Difficulties involved in measuring objects, especially irregular or small objects.
- How to define or describe diffusion.
- Qualitative descriptions of concentration and dilution.
- Methods of cell communication.
- Construct an investigative lab (optional)

Lesson Background: Diffusion is a naturally occurring process wherein atoms, molecules, nanoparticles, or other small bodies suspended in a background medium move from an area of high concentration to low concentration. The process of diffusion is usually considered to be slow from a macroscopic point of view. For example, if a bottle of perfume is opened at the front of a room, it may take many minutes for the molecules of the perfume vapor to diffuse to the back of the room where the scent can be detected. While the diffusion process is slow over the length scale of meters, at the nanometer scale diffusion becomes a dominant mode of chemical transport. Atoms, ions, and molecules may diffuse across a span of a few nanometers in a matter of milliseconds or faster.

This is relevant to nanotechnology as diffusion is an important process in cell signaling which occurs at the micro- and nanoscales. Adjacent nerve cells (neurons) pass electrical impulses by diffusion of chemical species across a gap junction, a distance of only 3.5 nanometers. Other cells utilize chemical exchange where cells are separated by a much greater distance of 30 nm. Cell may also employ chemical messaging in which chemicals diffuse throughout the entire cell traveling distances of a few micrometers to over 100 micrometers. (1)

Diffusion has many applications. For example, diffusion is essential for oxygen transport in our lungs and the removal of cellular respiration waste products from our lungs. Oxygen in air

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moves down its concentration gradient as it enters the cells in our lungs and the carbon dioxide generated from cellular respiration moves down its concentration gradient as it exits the cells in our lungs. Diffusion is also an important process in oxygenating the water in our lakes and other water systems. Many of the fish and other water dwelling organisms depend on this process to survive.

One consequence of the nanoscale importance of diffusion is that it is employed by cells as a way to communicate with surrounding cells and their environment; this process is known as *passive transport*. Cells use constraints such as particle size and charge to only allow certain chemical species to diffuse through their outer membrane. The rate at which a particle diffuses depends on a number of factors. In simple terms, the rate of a particle's diffusion is directly related to the *gradient* of particle concentration (that is, how the concentration changes with distance) and inversely related to the distance traveled. The relationship called Fick's Law is used to predict the diffusion rate. Fick's Law gives an expression for *diffusive* flux, that is, how many particles pass through a defined unit area per unit time. One form of Fick's (1^{st}) Law may be written as:

$$J = -D\frac{\Delta n}{\Delta x}$$

where *J* is the diffusive flux, *n* is the particle concentration (number of particles per unit volume), *x* is distance, and *D* is a constant called the *coefficient of diffusivity,* which is a property of the diffusing species. During the diffusion process, there may be a high concentration initially, but as the particles diffuse outward, the concentration will drop. Therefore, the diffusive flux is not constant and will slow down as the diffusing particles travel longer distances and become diluted in the process. Thus, cell signaling is slower in situations when diffusion occurs over long distances and a small concentration gradient exists.

Sources:

1. Truskey. A, Yuan Fan, Katz, F., Transport Phenomena in Biological Systems Second Edition, Pearson Education Inc., 2009.

Pre-requisite Knowledge: Students should understand solutions and concentration gradients.

Materials: (for each lab group of 3-4)

- A clear plastic thin stem pipet
- Metric ruler capable of measuring mm
- Food coloring (red, blue or green)
- Timer/stopwatch
- Aluminum foil
- Petri dish (to hold food coloring)
- Thermometer
- Beakers to hold cold and hot water
- Graph paper or spreadsheet program

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Advance Preparation. Purchase materials, most of which can be found in a household goods store or hardware store. The pipets used in this lab were purchased from the Flinn Scientific Catalog # AP1718. These are available at most scientific supply companies.

Vocabulary and Definitions: These should be reviewed before the activity begins or assigned as homework and then discussed in class to correct any misconceptions.

1. *Diffusion*: the net movement of a substance from a region of high concentration to a region of low concentration.
2. *Concentration*: a measurement of how much of a substance is in a given volume (drops of food coloring / ml of water)
3. *Dilution*: typically done by adding water to an aqueous solution.
4. *Rate*: the distance something travels in a given time (cm/sec). It is helpful to explain that rates are not usually constant. Explain the difference between instantaneous rate and average rate. The example of driving to school in the morning is helpful to understand this concept.
5. *Active transport:* is the movement of molecules or ions against a concentration gradient (from an area of lower to higher concentration). This does not ordinarily occur so such transport requires energy often carried out with the help of ATP, (an intracellular source of energy) and enzymes.
6. *Passive transport:* is carried out when the concentration or pressure differences influence movement. A concentration gradient exists when there is a difference in concentration between the inside and outside of the cell.
7. *Nanoscale*: measured in nanometers; typically referring to materials between 1 and 100 nm but others use up to several hundred nanometers.
8. *Nanometer*: $1x10^{-9}$ or one billionth of a meter.

Suggested Teaching Strategies:

Time	**Activity**	**Goal**
Day 1 15 min	Pre-Lab: show diffusion /osmosis video, introduce concentration and rate of diffusion**.** https://www.youtube.com/watch?v=GrDpaSfyfjo	To accurately view and model diffusion that takes place in cells. To measure the rate of linear diffusion and have a qualitative understanding of concentration.
Day 2	Conduct Lab	To measure rates of linear diffusion (cm/second) and determine relationship with distance, concentration gradient, and temperature.
5 min	Students answer warm-up questions… Diffusion, rate, concentration gradient.	To make sure students understand the concepts of diffusion, rate and concentration.
40 min	Distribute *Student Worksheets* to students. Students follow procedure	To allow students to explore rates of diffusion relative to distance, concentration gradients, and temperature.
5 min	Clean up.	To prepare workspace for next class.

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Guided Dialogue:
Ask students questions to provoke thought and review what they already know:

1. If I drop food dye into a pool, what would happen? *The dye would spread out until it is evenly mixed throughout the pool.*
2. How long would it take for the dye to spread from one end of the pool to the other assuming there was no pump moving the water and the pool was 10 feet long? *Answers will vary, there is not a "correct" answer to this question. Most students will guess several seconds or a few minutes.*
3. How long would it take if the pool was only 5 ft long? *Answers will vary, but most students will predict it to take half as long as it took to spread in the 10 ft pool.*
4. What do you think would make the food dye spread faster besides mixing the water with an outside force such as a pump or mixing with a stick? *Answers will vary; this is intended to get students thinking about possible research ideas for the optional inquiry based lab experiment.*

Troubleshooting Tips:
Remind students to make careful measurements throughout the lab and clean up any spills. Caution students that if they draw up the food coloring into the pipet too quickly it will shoot up and mix with the water in the pipet tip. They will then need to begin the experiment again. Remind them that measurements will be in centimeters.

Directions for the Activity:
Step by step directions are in the Student Guide with answers found below.

Follow up questions:

1. How would this experiment be more difficult if we were using actual cells instead of plastic pipets to represent the cells?. *Actual cells do not have a definite shape so we cannot simply measure their length. They are also very small so we would need special equipment to make the necessary measurements. We couldn't simply see the particles diffuse with our eyes so again we would need special equipment to detect how far the particles have diffused in a given time.*
2. How long do you think it takes for chemicals to diffuse between two cells? *This answer will vary, but this is a cool link to help think about how to answer this question.* *http://umdberg.pbworks.com/w/page/61013258/Relay%20cells* A more simple approach would involve setting up a ratio between the time it takes to diffuse the length of the pipet and compare it to the distance between two cells (3.5 nm). Ex: 12cm/3sec = 3.5nm/x sec. Remind students to be careful with units and make sure they convert nm to cm correctly before doing the calculation.

Assessment: Students should be able to show understanding of these qualitative concepts.

1. Describe diffusion using words or pictures.
2. Explain concentration qualitatively (how many times more concentrated or dilute).
3. Use data to construct a plot of diffusion time vs. distance traveled.

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4. Use the slope of the plot to determine rate of diffusion.
5. Identify diffusion rate as linear (constant rate) or non-linear (changing rate).
6. Explain the role of diffusion in cell signaling.

Resources: You may wish to use these resources either as background or as a resource for students to use in their inquiry-based design.

- Wikipedia. "Diffusion." (accessed February 2015) http://en.wikipedia.org/wiki/Diffusion
- Science Clarified. "Osmosis – Real-life applications." (accesses February 2015) http://www.scienceclarified.com/everyday/Real-Life-Chemistry-Vol-2/Osmosis-Real-life-applications.html
- Wikipedia. Fick's Law of Diffusion: https://en.wikipedia.org/wiki/Fick%27s_laws_of_diffusion
- Cell Signaling: https://en.wikipedia.org/wiki/Cell_signaling
- Introduction to Cell Signaling: https://www.khanacademy.org/science/biology/cell-signaling/mechanisms-of-cell-signaling/a/introduction-to-cell-signaling
- Nano 101: https://www.nano.gov/nanotech-101
- An Introduction to Nanotechnology: From Understanding Nano: https://www.understandingnano.com/introduction.html
- YouTube; Fuse School: Diffusion, Osmosis, and Active Transport Part 1: https://www.youtube.com/watch?v=PRi6uHDKeW4 and Part 2: https://www.youtube.com/watch?v=eDeCgTRFCbA

Next Generation Science Standards:

- HS.LS.1A: Structure and function
- HS.LS1-2: Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions with multicellular organisms.
- Science and Engineering Practices:
    - Developing and using models

Optional Activity Extension:
Design an experiment to test other variables that you would like to examine their movement. What other variables would you test? Would you want to use the same pipet to observe diffusion or would you change the shape of the container? What type of molecules would you examine? Large versus small? Vitamins, minerals, salts? What about the medium through which your materials will diffuse? Design your experiment and have the instructor approve your design before testing. Write up your results and then share as a short presentation to the class.

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Student worksheet

(with answers in red)

Linear Diffusion and Cell Signaling

Introduction:

Your ability to read these words is dependent on cell communication. A stimulus (light) reaches the cells in your eyes and those cells propagate (spread) that signal to the cells next to them until the signal reaches your brain. Your brain then translates that signal into letters and words. This process is quite complex, but an important part of this process is dependent on diffusion. Diffusion occurs when one cell releases chemicals into the interstitial fluid (the stuff surrounding the cells) and those chemicals diffuse (spread) to neighboring cells. This process is repeated until the signal reaches your brain. The faster your brain receives the signal, the faster you are able to react to a given stimulus. Drag car racers need to react very quickly to the stimulus of a green light. The driver who processes the stimulus faster gets a “head start” over the other racer.

Diffusion is a naturally occurring process wherein atoms, molecules, nanoparticles, or other small bodies suspended in a background medium move from an area of high concentration to low concentration. The process of diffusion is usually considered to be slow from a macroscopic point of view. While the diffusion process is slow over the length scale of meters, at the nanometer scale diffusion becomes a dominant mode of chemical transport. Atoms, ions, and molecules may diffuse across a span of a few nanometers in a matter of milliseconds or faster. This is relevant to nanotechnology as diffusion is an important process in cell signaling which occurs at the micro- and nanoscales.

In this lab you will explore the concept of diffusion on a macro scale to gain a better understanding of how the cells in your body communicate on the micro and nano scale. Furthermore, you will identify several factors that influence the rate of diffusion and use those factors to postulate ways our body could increase the rate of cell signaling.

Prelab Questions:

1. What will happen to the rate of diffusion if we double the distance it has to travel?

Most students will predict that the amount of time will double or that the rate will stay the same. However, we should see that it actually takes more than twice as long to travel twice the distance, therefore the rate should decrease as distance increases.

2. What are three things that might make diffusion occur faster?

In theory, increasing temperature and concentration and decreasing distance traveled will lead to an increase the rate of diffusion.

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Materials: (for each lab group of 3-4)

- A clear plastic thin stem pipet
- Metric ruler capable of measuring mm
- Food coloring (red, blue or green)
- Timer/stopwatch
- Aluminum foil
- Petri dish (to hold food coloring)
- Thermometer
- Beakers to hold cold and hot water
- Graph paper or spreadsheet program

Procedure:

You will determine the rate of diffusion for food dye in a thin stem pipet filled with water. The pipet is a macro-model of cell diffusion which occurs at the micro/nano-scale. You will vary the concentration and temperature and compare results with the control.

1. Fill a plastic pipet with water so that the bulb and stem **are completely filled** with water.
2. Hold the pipet so the stem is pointing down and carefully squeeze out **one** drop of water. When you release the bulb there should now be a small amount of air in the tip of the pipet.
3. Place two drops of food coloring on a small piece of aluminum foil or some other non-absorbent material such as a petri dish.
4. With the pipet tip facing down, carefully squeeze the pipet bulb so that the air is evacuated from the tip of the pipet.
5. Have a stop watch or timer ready.
6. Place the tip of the pipet into the food coloring and very slowly "draw-up" the food dye into the tip (**NOTE**: if done too quickly, the food dye will shoot up and mix with the water in the pipet tip; if this happens, begin again).
7. Lay the pipet down on its side next to the ruler so that the top of the food dye is at zero on the ruler. Record how far the dye travels in 20 second intervals. Record each distance traveled in cm and report to the nearest tenth place (ex: 1.5 cm). You may use the data table below or your lab notebook – teacher will tell you which one to use.
8. Record the distance for 10 minutes (30 total data points!)
9. Repeat the experiment in hot or cold water and record the temperature in degrees Celsius.
10. Repeat the experiment after diluting the food dye to half its original concentration.
11. Graph your data for each experiment.

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Data Table:

Time (Min:sec)	Diffusion Distance		
	Food Dye	Diluted Food Dye	Hot/Cold Water ____°C
0:00	0.0 (cm)	0.0 (cm)	0.0 (cm)
0:20			
0:40			
1:00			
1:20			
1:40			
2:00			
2:20			
2:40			
3:00			
3:20			
3:40			
4:00			
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8:40			
9:00			
9:20			
9:40			
10:00			

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Analyze the Results:

Plot all three sets of data on the same graph with time on the "x" axis and distance on the "y" axis. Be sure to use appropriate labels and units. Answer the questions below based on your results:

1. Is your data linear or not? What does this tell you about the rate of diffusion?

If the data is linear for each trial, this suggests that the rate is constant and does not change as the dye diffuses through the straw. If the data is curved and there is a decrease in the slope as the dye travels through the straw this suggests that the rate decreases as it travels through the straw. The rate should not increase as the dye travels through the straw.

2. Which trial had the fastest rate? Which trial had the slowest rate? Does this make sense? Why or why not?

In theory the hot water should diffuse fastest than the control, and the cold water and diluted food dye should diffuse slower than the control.

3. When cells communicate with their neighboring cells, they release chemicals which diffuse through the interstitial fluid that separates them. What would allow these cells to communicate more quickly (think about distance, temperature, and concentration).

If the cells were very close together, in a warm environment, and released a high concentration of chemicals, they would diffuse faster to their neighboring cells.

4. If the distance between two cells is 35 nanometers, how long would it take for the chemicals released from one cell to diffuse to the next cell? (Assume a constant rate of diffusion and use the rate determined from your trial with the control.

Set up a ratio using your data. For example: 10 min/4.5 cm = x/35nm. Of course students will need to convert the distance units so they cancel out in this calculation.

5. Why is diffusion and cell signaling considered a nanoscale phenomenon?

Answers will vary but should include that at the cellular level atoms, ions, and molecules are at the nanoscale and may diffuse across a span of a few nanometers.

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Going Further: Your instructor may require you to answer these challenge questions:

1. How would this experiment be more difficult if we were using actual cells instead of plastic pipets to represent the cells?. *Actual cells do not have a definite shape so we cannot simply measure their length. They are also very small so we would need special equipment to make the necessary measurements. We couldn't simply see the particles diffuse with our eyes so again we would need special equipment to detect how far the particles have diffused in a given time.*
2. How long do you think it takes for chemicals to diffuse between two cells? *This answer will vary, but this is a cool link to help think about how to answer this question.* *http://umdberg.pbworks.com/w/page/61013258/Relay%20cells*
   A more simple approach would involve setting up a ratio between the time it takes to diffuse the length of the pipet and compare it to the distance between two cells (3.5 nm). Ex: 12cm/3sec = 3.5nm/x sec. Remind students to be careful with units and make sure they convert nm to cm correctly before doing the calculation.

Cleanup: Follow your teacher's instruction. Used food coloring can be safely dumped down the drain. Discard aluminum foil or wash petri dish is used. Pipets may be discarded.

Optional Activity Extension:
Design an experiment to test other variables that you would like to examine their movement. What other variables would you test? Would you want to use the same pipet to observe diffusion or would you change the shape of the container? What type of molecules would you examine? Large versus small? Vitamins, minerals, salts? What about the medium through which your materials will diffuse? Design your experiment and have the instructor approve your design before testing. Write up your results and then share as a short presentation to the class.

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