Teacher's Guide

Visualizing Diffusion in Real Time and on a Macroscale

Grade Level: High school

Subject area: Chemistry & Biology

Time required: 60 minutes

Learning Objectives: Through observation and interaction students will observe diffusion rates, comprehend the importance of atomic radius on diffusion, and understand concentration

Summary: This lesson has been designed to help students visualize how diffusion occurs by creating a macro version of a permeable membrane. The goal is to have students see how molecular size (atomic radii) impact diffusion at the nanoscale. In addition, they will observe diffusion rates of iodine with varying percentages of agarose solutions. These two activities will be used to introduce students to diffusion and evaluate their understanding of the concepts of diffusion and density. The lesson also is designed to help students better comprehend the interaction of molecules on the nanoscale.

Lesson Background: The concept of scale and diffusion are topics that students frequently struggle to comprehend and apply. For the students, this is due to the "invisible" or "mythical" nature of the subjects as they take place at the micro and nanoscale. To make them tangible, this exercise will allow students to visualize diffusion at these scales by using a macroscale model which demonstrates diffusion in real time.

Diffusion is the movement of molecules down a concentration gradient, which is effected by a number of characteristics (kinetic energy, crystalline structure, atomic radii, etc.). It is further complicated when diffusion occurs through another substance. For this exercise, two of the variables that are discussed are the atomic radii of molecules and the substances through which they are diffusing. Understanding diffusion is easier when comparing the atomic radius of varying elements and molecules: those with smaller atomic radii are more easily able to diffuse through a substance and vice versa. Additionally, as the radius of the substance through which something is diffusing increases, the diffusion rate decreases. The larger the element or molecule, the more difficult it is to move across a membrane or materials. Agarose plates, containing starch, of varying agarose percentages will be used to allow students to observe diffusion through varying medium. The starch in the agarose will interact with the diffusing molecule, iodine, while being too large to diffuse through the medium itself. As the iodine diffuses through the agarose and interacts with the carbohydrate in the starch, the dramatic change in color (with the starch) will allow students to observe, in real time, the dispersion of iodine. Depending on the agarose percentage being observed, the iodine will migrate more quickly (in lower % agarose) or slowly (in higher % agarose). This is due to the fact that in the denser plates iodine atoms will have more numerous interactions, or "collisions", with the agarose which effects its rate of diffusion.

The characteristics of nanoparticles vary greatly from those of their macro-scale counterparts due to the dramatic difference in surface area to volume ratios. To better understand this small scale, a nanoscale representative is "blown-up" to the macroscale, with some obvious liberties. This model allows student to tangibly observe "nanoscale" interactions and relate these observations to abstract concepts covered in class.
While we know that diffusion is an important part of cell function, it is also an important research aspect in nanotechnology. One area that scientists and engineers actively explore is diffusion and thin films. Thin films are layers of materials on the order of nanometers to micrometers. Common applications are thin film solar cells, thin film batteries, drug delivery, surface coatings and more.

Resources:

- Size and Scale: https://www.nnci.net/node/5305
- Size and Scale: NanoSense Size MatterLesson 2: http://nanosense.sri.com/activities/sizematters/sizeandscale/SM_Lesson2Teacher.pdf (answers) and http://nanosense.sri.com/activities/sizematters/sizeandscale/SM_Lesson2Student.pdf(images).
- Introduction to Nanotechnology: https://www.nano.gov/nanotech-101
- Starch Iodine Diffusion Labs: There are several online examples of this lesson as well as YouTube videos. One example - https://www.biologycorner.com/2009/09/16/diffusion-lab/

Pre-requisite Knowledge: Students should have a knowledge of concentration and concentration gradients. They should also know what an atomic radius is. Students should kow that iodine is an indicator of starch.

Vocabulary:

Diffusion **–** the net movement of molecules or atoms from a region of higher concentration (or high chemical potential) to a region of lower concentration (or low chemical potential). Diffusion is driven by a gradient in chemical potential of the diffusing species. (https://en.wikipedia.org/wiki/Diffusion)

Concentration gradient - this occurs where the concentration of a substance or material changes over a particular distance. Typically, the substance diffuses from its highest concentration to its lowest concentration. Think of room diffusers where the scent is highly concentrated in the container and moves outward into the room's air.

Atomic radius – a measure of the size of a chemical element's atoms. Because an atom's boundary is not well defined the radius measured as the mean or typical distance from the center nucleus to the boundary of the orbiting electrons.

Nanoscale - a nanometer is one billionth of a meter or 10^{-9}m. Materials of this size are considered to be on the nanoscale. To see how small this is visit: https://www.nano.gov/nanotech-101/what/nano-size

Nanotechnology - Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications occur.
Nanoparticle – a particle that is typically between 1 and 100nm.

Materials: (per group of 2)

- Large Dish or Tray
- Three (3) Starch Agar Plates of varying agar % (Recipe in Advanced Preparation)
    - Recipe
        - Agar
        - Distilled Water
        - Petri Dishes
        - Starch
    - Plate Percentages
        - 2% Agarose
        - 1% Agarose
        - 0.5% Agarose
- Iodine Solution
- Ruler (cm)and scissors
- Pipet or dropper
- Macro-scale nanoparticles setup (Directions in Advanced Preparation)
    - 6" in diameter; 2' in length
    - Tennis balls (~30)
    - Nylon string
    - Plastic bowl with 6" base
- Series of 4 spheres or atom representatives
    - Plastic Air soft Pellets or Metal BB's (0.44 cm)
    - Marbles (~1.5 cm)
    - Golf Balls (4.27 cm)
    - racquetballs (5.7 cm)
- Empty box to catch balls
- Plastic bag to mix "atoms"

Safety Information: Care should be taken while cutting the inner circle of the agarose plates. Iodine can stain so caution students to not touch the liquid.
Vocabulary and Definitions

Advance Preparation:

1. Assembly of Macro-Scale Nanoparticle Apparatus
    a. As seen in Figure 1 below four tennis balls are inserted into the bottom of a 6" in diameter, 2 foot long section of PVC tubing (clear is best).
    b. Holes are drilled 2 inches from the bottom of the tubing and the center of each tennis ball. Drill in each tube.

c. Nylon string is inserted through the tennis balls and knots are make on the outside of the PVC to anchor them in place.
d. Additional layers of tennis balls (four) are subsequently stacked on top until they reach to top of the PVC pipe.
e. Again, for the top layer drill holes through the tubing and tennis balls and secure in place using nylon string.
f. To create a funnel, the bottom of a large bowl was cut out and the bowl was then glued to the top of the 6" PVC tube as seen in Figure 2 below.

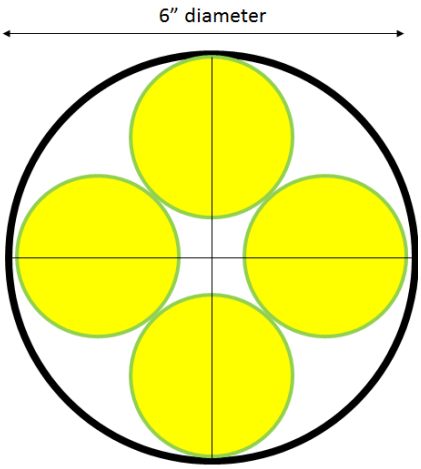

Figure 1

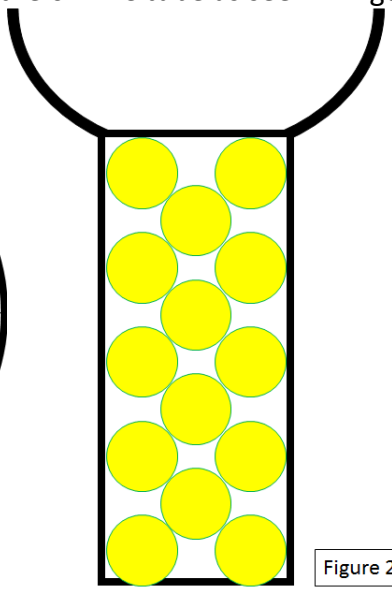

Figure 2

2. In a gallon or grocery bag mix together the plastic air soft pellets, marbles, golf balls and racquetballs thoroughly. This will represent a mixture of atoms of varying atomic radii.
3. Preparation of starch agar plates (Three different preparations need to be made to make agar plates of varying percent agarose).
    a. Mix together agar and starch in the following concentrations:
    b. 2% Agar Plates - 10 g Agar
    c. 1% Agar Plates - 5 g Agar
    d. 0.5 % Agar Plates - 2.5 g Agar
    e. Add 500 ml of distilled water to each mixture
    f. Heat to near boiling, while continuing to stir
    g. Remove from heat and allow to cool until the container can be handled
    h. Pour plates and allow them to cool and solidify
    i. Store plates upside down and use within 48 hours
    j. This recipe makes ~20 plates of each concentration

Troubleshooting Tips: Instruct the students to not overfill the central hole in the agarose plates. Before performing the lab, students must address the "Pre-lab" questions (see student worksheets below).

Suggested Teaching Strategies: During the introduction to the activity, the general concepts of diffusion and concentration gradients should be reviewed. At this time scale, conversion factors, and diffusion are terms and concepts that should be reviewed before the activity begins.

Time	Activity	Goal
5-10 Min.	Introduction	Introduce the activity and broadly cover general topics. Break class into two groups.
20-30 Min.	First Rotation	One half of the class will work on part of the lab (investigating the concept of diffusion) and the other half will work on the second concept (understanding nano-scale).
2 Min.	Transition	Switching groups
20-30 Min.	Second Rotation	One half of the class will work on part of the lab (investigating the concept of diffusion) and the other half will work on the second concept (understanding nano-scale).

Procedure for the Activity:
After introducing the lab and concepts, divide the class into two groups and, further, into partners. As there are two parts to this lab, only half of the time is allocated for one part. (For clarity, from this point on the half working on investigating the concept of diffusion will be **Group A** and the half working on understanding nano-scale will be **Group B**) Each group will work ~20 minutes and then rotate to the other group.

Group A Procedure:

- Each partner pair will be given a tray with three starch agar plates (2%, 1%, 0.5%), iodine solution and a ruler (preparing lab station ahead of time is recommended).
- Students will cut out and dispose of a 1" in diameter circle of agarose from the center of each plate.
- Next they will use a dropper to fill the 1" voided center of each plate with iodine and wait 10 minutes.
- While waiting, students should be answering the "Lab Prediction" questions (see student worksheets below).
- At the end of the 10 minute period, the students will measure the migration distance of iodine from the center of each plate. Iodine will interact with the starch in the agar leading to a black color. The iodine SHOULD diffuse farthest through the 0.5% agarose plates.
- Time permitting students can work on some of the pertaining "Post-Lab" questions (see student worksheets below).

Group B Procedure:

- Each partner pair will be given an empty box, a "Macro-scale Nanoparticle Setup" and a bag of "Atom Representatives" (see advanced preparation for instructions).
- While one partner holds the Macro-scale Nanoparticle Setup over the empty box, the other will shake and pour the contents of the bag into the top.

- Once all the “atoms” have passed through the apparatus, students will evaluate which atoms were able to “diffuse” through and which ones did not. The smallest “atoms”, the plastic air soft pellets and marbles, should be the only ones that the students find in the box.
- Time permitting students can work on some of the pertaining “Post-Lab” questions (see student worksheets below).

Assessment: Once the two activities are completed, there should be a class discussion of the results and post lab questions. This will be a good time to address misconceptions regarding diffusion and concentration gradients. Assessment will be the student answers on the post lab questions. A possible rubric is included with this lesson.

Next Generation Science Standards:

- HS-PS1 Matter and its interactions
  - HS-PS1-1 Use a model to predict the relationship between systems or between components of a system
- HS-PS1A Structure and properties of matter
- HS-PS1B Chemical reactions
- HS-PS2-6 Communicate scientific and technical information about why the molecular-level structure is important in the functioning of designed materials
- HS-LS1A Structure and Function

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Student Worksheet (with answers in red)

NAME: _____ PERIOD: _____ DATE: _____

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Part A

Answer the pre-lab questions:

1. Define diffusion. Describe one example of how it is important to life.
   Diffusion is the movement of molecules from an area of high concentration to an area of low concentration.
   Importance: Gas exchange in cells (O_2), glucose distribution, waste removal etc.
2. What determines whether or not something can diffuse across a membrane? Explain
   Atomic radius, kinetic energy (temperature), concentration
3. What macromolecule does iodine test for? What indicates a positive reaction?
   Starch. Turning black(deep purple) indicates a positive reaction.

Lab Prediction Questions:

1. Given the three concentrations of agarose plates used (2%, 1%, and 0.5%), predict which one the iodine will diffuse through the quickest. Explain your choice.
   Iodine should move through the 0.5% agarose gel quickest since there are less agarose molecules for the iodine to interact with, and therefore slow them down.
2. What is going to happen to the clear agarose as the iodine diffuses through it? Why?
   It will turn black as it interacts with the starch in the gel. Molecules of iodine moved from the center to the plate containing starch and the black color is an indicator of starch molecules. Iodine is a starch indicator.
3. Why do you predict that the iodine will move towards the agarose plates? How can this happen?
   The iodine moves from a higher concentration to a lower concertation (concentration gradient). The iodine molecules are smaller than the agarose plate material (starch) and can diffuse into the plate.
4. Will the central well of iodine change color? Explain.
   No. The starch in the gel is TOO LARGE to diffuse out of the agarose thus cannot move into the central iodine well.

Materials:

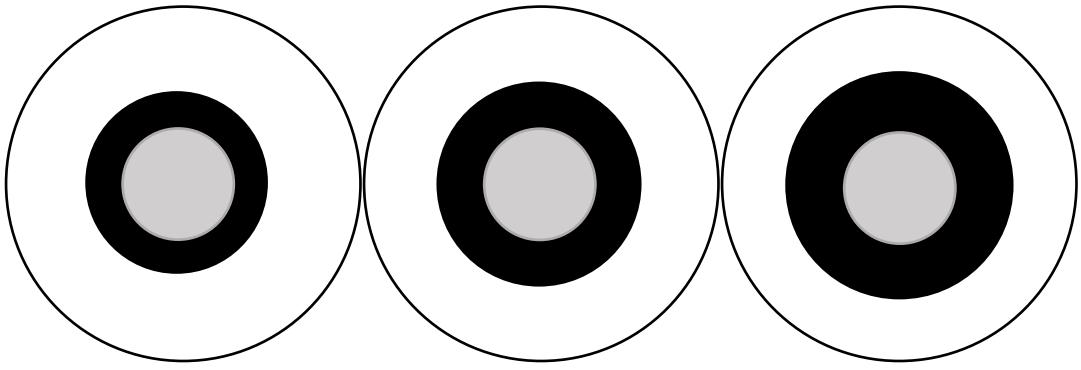
- Large Dish or Tray
- Three (3) Starch Agar Plates (2%, 1% and 0.5%)
- Iodine Solution
- Dropper/pipet
- Ruler
- Scoopula/scissors

Procedure:

1. Each partner pair will be given a tray with three starch agar plates (2%, 1%, and 0.5%), iodine solution and a ruler.
2. Cut out a 1" in diameter circle of agarose from the center of each plate using a scoopula/scissors. Discard the cut out portion in the trash.
3. Using a dropper, fill the 1 inch void in the center of each plate with iodine (DO NOT OVERFILL so that any is outside the center) and wait 10 minutes.
4. While waiting for the reaction, answer the "Lab Prediction" questions. Answer them in complete sentences and provide explanations.
5. At the end of the 10 minute period, measure the migration distance of iodine from the center of each plate in centimeters. Record data in the results table on the back.
6. Sketch what your agarose plates look like after the 10 minute period. This should be done in the circles in the results section of the worksheet. Make sure to label and color each appropriately.
7. Properly dispose of your agarose plates and clean up the lab stations per your teacher's instructions.
8. With any time remaining work, with your partner, on the post-lab questions.

Results:

Agarose Concentration	Distance of Iodine Diffusion (in cm)
2% Agarose	0.75 cm
1% Agarose	1.5 cm
0.5% Agarose	3 cm



Label iodine central circle, iodine diffused into plate, agarose plate and percentages

Post-Lab Questions:

1. Did the agarose percentage effect how far the iodine could move through the plate? What would have happened if you had a 0.25% plate? Why?

   It did, with the lowest percentage showing the furthest movement from the central well. The iodine would diffuse farther in a 0.25% plate because the iodine atoms will have less starch atoms to interact with, allowing for faster diffusion.

2. Did the central iodine well turn colors? Why or why not? (Hint: Size matters!) When would it have changed?

   No. The starch molecules are too large to diffuse through the agarose into the central well. The starch molecule would need to be smaller in order to diffuse.

3. What is the difference between the agarose plates? What does this have to do with the ability for substances to diffuse through it?

   The difference is how close the agarose strands are (how dense). This means the iodine molecules interact with or "bumps into" more agarose molecules thus slowing down diffusion. Likewise, more agarose means smaller "gaps" between molecules. With increasing percentage of agarose the density increases.

4. Provide an example of how the properties of diffusion observed today apply to the properties of diffusion in animal cells.

   [Numerous examples will work] The density of a cell membrane (due to amount of cholesterol, protein composition, or temperature) will affect the rate and ability for molecules to diffuse across it.

5. If the iodine and starch were switched (agarose plates were made with iodine and starch was poured into the 1 inch center of the plate) would the results be the same? Why or why not?

   No because the starch molecules are too large to pass into the plate but iodine could pass into the starch center because of the concentration gradient and their smaller molecular size.

NAME: _____ PERIOD: _____ DATE: _____

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Part B

Pre-lab Questions:

1. How is a nanoparticle defined?
   Any particle <1nm-100nm in size.
2. What determines if something will diffuse through a membrane or substrate?
   Atomic radius of diffusing particle and substrate.
3. What shape do we consider atoms to have? Give an example of something, on a large scale that has a similar shape.
   Spherical in shape. Ball, globe, bowling ball, etc.

Materials:

- Macroscale Nanoparticles apparatus
- Bag of:
  - Plastic Air soft Pellets or Metal BB's
  - Marbles
  - Golf Balls
  - racquetballs
- Medium sized box
- Ruler

Procedure:

1. Measure the diameter of each of the different types of spheres you are given, and record the data in the results section. Each of these sphere types will represent atoms of various radii.

2. While one partner holds the Macroscale Nanoparticle Apparatus over the empty box, the other partner will shake and pour the contents of the bag into the top.

3. Allow all the "atoms" to flow down through the apparatus. Evaluate which ones were able to "diffuse" through and which did not. Record how many of each type of "atom" made it through to the box.

4. Repeat step 3 and compare the results of both trials.

5. Put the spheres back in the original bag and return all the materials to the original lab space.

6. With any time remaining work, with your partner on the post-lab questions.

Results:

Type	Size (in cm)	Starting Number of Each	Number of Each in Box	Did "diffusion" occur (Y/N)
Racquetball	5.6 cm	6	0	N
Golf Ball	4.2 cm	8	0	N
Marbles	1.4 cm	10	10	Y
BB's	0.6 cm	12	12	Y

Post-lab questions:

1. What does the "Macroscale Nanoparticles Apparatus", along with the activity, represent? Using complete sentences explain your reasoning.
   It represents a solid substrate through which particles can diffuse. Depending on the atomic size of the substrate, varying molecules can diffuse through it.
2. Which spheres were able to "diffuse" through the apparatus? Which were unable to? Explain why each were/weren't able to.
   The BB's and marbles were able to diffuse the apparatus into the box. Golf balls and racquetballs were unable to. Each was/wasn't able to due to interference from substrate atomic radii size.
3. What determines if an atom/molecule can diffuse through a substance?
   Whether substrate radii are large enough and diffusing particle radii are small enough, i.e. the diffusing particles (molecules) are smaller than the "openings" in the substrate material allowing them to pass through with hinderance.
4. Using what you know, how can the process you observed in this exercise be used in the "real world"? What are potential applications?
   Storing of particles within substrates. Size specific selection of particles. Movement of materials into or out of a cell. Creation of impermeable or permeable materials.
5. What is nanoscale about this activity?
   The diffusion occurs at the molecular and atomic level. The atomic radius is at the nanoscale.

Challenge:

Assume the spheres represent individual atoms and the tennis balls in the apparatus represent cesium which has a radius of 298 pm. Using an atomic radius chart and a ruler, what elements would the other spheres represent? What is the largest and smallest atoms/molecules that could diffuse throughout the setup?

Sphere	Size (cm)	Size (pm)	Element
Tennis ball →			
Racquetball →			
Golf ball →			
Marble →			
BB's →			