Lesson Plan: Diffusion

Background

Particles in cells show rapid back and forth movement, or **Brownian motion,** which is also known as **diffusion**. The back and forth motion consists of random steps from a starting position, resembling a random walk. Diffusion is faster at higher temperatures and for smaller particles. Particles show diffusional movement not only in cells, but also in water and other fluids. This lesson plan demonstrates diffusion in gels, which are networks of cross-linked molecules in water or other fluids. Diffusion in gels, as well as in viscous liquids, is slower than in water. The cytoplasm in cells is very thick and viscous – it flows very slowly. The high viscosity of the cytoplasm causes particles in cells to diffuse more slowly than particles diffusing in water.

This laboratory shows that diffusion of molecules occurs in gels. It also shows that molecules diffuse faster at higher temperature and small molecules diffuse faster than larger ones. By measuring and plotting the diffusion distance over time, advanced students will be able to show that there is a linear relationship between the diffusion distance squared and diffusion time.

Objectives & Grade Level

Demonstrate diffusion in 2D and the effects of temperature, molecular weight (*MW*), and diffusion distance on diffusion time. Appropriate for beginning to advanced high school science classes; see notes for advanced students.

Materials

- Agarose or Gelatin
  Jello (lemon-flavored for light background color) can also be used
- Optional: Sugar
  Sucrose is the same as Sugar and can be used instead of Sugar
- Potassium Permanganate or Methylene Blue Dye
  Food dyes can be used instead, e.g., FD&C Blue 2/Indigo Blue (263 MW); other food dyes are higher MW or mixtures of two dyes, e.g., McCormick Green Food Color is a mixture of FD&C Blue 1 (793 MW) and FD&C Yellow 5 (534 MW) – if you use this dye in a diffusion experiment, it should show a halo of yellow around a green dye spot with a blue-green center
- Petri dishes with covers
  Other small flat containers can be used instead, e.g., lids, dishes or shallow bowls
- Pipetman with tips or Pasteur pipette
  Round wooden toothpicks can be used instead to make a hole in the gel and add the dye to the gel
- Timer
- Disposable gloves, Goggles, Hot pads/mitts

Preparation of Materials

1. Make solutions of the dyes in water: Potassium Permanganate (0.16 *g* in 10 *ml* = 100 *mM*)
   Methylene Blue (0.32 *g* in 10 *ml* = 100 *mM*)

   Caution: use disposable gloves and wear goggles when weighing out dye powders. *The concentration should be ~100 mM; the exact concentration is not critical. Solutions can be made, tightly capped, and kept at room temperature for several months or longer. Food dyes can be used from the bottle.*

2. Add enough agarose or gelatin to a flask or beaker to make a final solution of 1% agarose or 3% gelatin in water (1 *g* in 100 *ml* = 1%).

   You will need ~30 ml of solution for a standard 100 x 15 mm petri dish or ~12 ml for a 60 x 15 mm petri dish. The volume can be increased so it is sufficient to pour 4-8 or more plates.

3. Carefully heat half of the water in a microwave oven or on a hot plate until hot and almost boiling; add to the agarose or gelatin in the flask or beaker and stir until dissolved.

   Caution: use hot pads/mitts, wear goggles, and avoid spills! *Heating the water will take 30-40 seconds on high in a microwave oven or a few minutes on a hot plate; heat until almost boiling.*

4. Add the rest of the water, which should be cold, and stir or swirl to mix. Let the solution cool a few minutes longer, then pour the plates to a depth of ~ 0.8 *cm*. Put the covers on the plates and let them stand at room temperature (agarose plates) or in a refrigerator (gelatin plates) without moving until set. The agarose plates will be ready to use in ~10 minutes and the gelatin plates will be ready in ~20-30 minutes.

   Plates can be made, tightly wrapped, and stored in a plastic bag in the refrigerator overnight, or longer (up to 4-6 weeks). For Jello, follow the instructions on the package and use the volumes above for each petri dish. Note that one cup (US) is ~237 ml; one package of Jello will make ~16-17 standard-sized plates. For ~8 plates, dissolve half a package (42.5 g) of Jello in half a cup (118.5 ml) of hot, almost boiling water, stir to dissolve, then add half a cup of cold water, swirl to mix, and pour the plates. Place the plates in a refrigerator for 1-2 hours or longer to set. For firmer Jello plates, reduce the water by half, i.e., dissolve one package of Jello in one cup of hot, almost boiling water, mix thoroughly and pour the plates. Let set ~20-30 minutes in a refrigerator. We call these 2x Jello plates.

Procedure

1. Diffusion of particles or molecules is due to heat, or thermal energy, which excites the particles or molecules, causing them to move. When they move, they collide with other particles or molecules – this results in random back and forth movement. We can observe diffusional movement using dyes – if we add a drop of dye to an agarose or gelatin plate at a given position, it will slowly spread out because the dye molecules are moving randomly from their starting position in the gel. You can see this movement if you do the following:

Take a petri dish of agarose (or gelatin or Jello) that has been sitting at room temperature for 5-10 minutes. Using a cut-off pipette tip or a glass Pasteur pipette, make a small hole in the agarose for each dye (**Figure 1**). You can also use a round wooden toothpick to make a hole, but the hole will be smaller. The position of the hole in the plate is not important, but it should be far enough from the edge of the plate so diffusion of the spot can be easily observed.

Carefully pipette a small drop (2-10 μl) of dye solution into the hole using a Pipetman or Pasteur pipette. Try not to overfill the hole or you will be observing flow of the dye over the gel surface, rather than diffusion of the dye in the gel. You can also add the dye to the hole by soaking a round wooden toothpick in dye for a few minutes, then inserting the toothpick into a hole made with another toothpick. Make sure the dye has transferred into the gel before removing the toothpick.

Then cover the petri dish and measure the diameter of the dye spot at different times after adding the dye, e.g., 0, 5, 10, 30, 60, 150 minutes (**Figures 2 and 3**). The increase in size of the dye spot will be easy to see even after 5 or 10 minutes. After measuring the diameter of the dye spot, divide by two to find the radius – the radius of the dye spot at each time is the distance the dye molecules have diffused from their starting position. Measure just to the edge of the dark dye spot which contains most of the dye molecules.

Photos can be taken at each time point using a cell phone. By including a ruler in the photo, you can accurately determine the diffusion distance from the photos – this is done by measuring the diameter of the dye spot in each photo and dividing by the length of one *cm* of the ruler in the photo to obtain the actual diameter of the dye spot; then divide the actual diameter by two to find the radius of the dye spot at each time point.

Are the times required for Potassium Permanganate and Methylene Blue to diffuse a given distance, e.g., 0.5 cm, in agarose (or gelatin or Jello) the same or different for the two dyes? If the times are different, which dye diffuses faster? Is the molecular weight of the faster dye larger or smaller than the molecular weight of the slower dye?

2. Warm a petri dish of agarose (or gelatin or 2x Jello) by placing it in an incubator at 37°*C* or holding it between your hands for 3-4 minutes and repeat the dye diffusion experiment. Keep the petri dish warm between time points by placing it in an incubator or holding it in your hands, keeping it horizontal.

Are the diffusion times for the dyes the same or different in the warm agarose (or gelatin or 2x Jello) compared to room temperature? If the diffusion times are different, are they faster or slower than at room temperature? Note that 2x Jello plates are recommended for this experiment, because they are firmer than normal Jello plates.

3. Cool a petri dish of agarose (or gelatin or Jello) by placing it in a refrigerator or on ice and repeat the dye diffusion experiment. Keep the petri dish cold between time points and make sure that it is horizontal.

Are the diffusion times for the dyes the same or different in the cold agarose (or gelatin or Jello) compared to room temperature? If the diffusion times are different, are they faster or slower than at room temperature?

4. Optional experiment: make 1% agarose (or 3% gelatin) plates that contain 20% sugar by adding 100 *ml* of hot, almost boiling water to 1 *g* of agarose (or 3 *g* of gelatin) + 20 *g* sugar in a flask or beaker, stir to dissolve, then pour the plates, as when making plates without sugar. Repeat the dye diffusion experiment at room temperature using these plates.

Are the diffusion times for the dyes the same or different in agarose (or gelatin) with 20% sugar as without sugar? If the diffusion times are different, are they faster or slower in the gel containing sugar?

Notes

1. Diffusional movement of single molecules or particles consists of random back and forth steps. In order to obtain information about diffusional movement of a given molecule, we need to measure and average the movements of many molecules over time. In these experiments, each dye spot consists of many millions of dye molecules. By measuring the increase in dye spot size with time in these experiments, we can obtain information about the average diffusional movement of millions of molecules over time (see Rob Phillips Group, 2005). The increase in size (or spreading) of the dye spot in the gel is caused by the average diffusional movement of the dye molecules from their starting position in the gel.

Note that molecules or particles also diffuse in water and other liquids, but diffusion alone, without the effects of convection currents and gravity, is very difficult to demonstrate in liquids (see Elster, 2014). This is because convection currents, which are movements of a liquid within another liquid, are created by simply adding a dye solution to the liquid. Besides forming convection currents, the higher density of the dye solution compared to water and other liquids causes it to gradually (or quickly) sink when it is added to these liquids. These movements of the dye in the liquid are not caused by diffusion. Because these movements and their effects are large compared to the diffusional movement of the dye, it is difficult to measure movement due only to diffusion by adding a dye solution to a liquid. In this lesson plan, we use agarose, gelatin or Jello plates because the gel prevents convection currents; we also measure 2D diffusion of the dye into the gel to avoid the effects of movements due to gravity.

2. Diffusion is faster for small molecules, e.g., Potassium Permanganate, than larger ones, such as Methylene Blue (**Figure 4**). The table gives the molecular weight (*MW*) and molecular formulae for the two dyes:

	MW	Formula
Potassium Permanganate	158 *g/mole*	$KMnO_4$
Methylene Blue	320 *g/mole*	$C_{16}H_{18}ClN_3S$

3. Diffusion times are dependent on temperature – they are faster in warm agarose (or gelatin or Jello) than room temperature plates, and slower in cold plates.

4. Gels are networks of cross-linked molecules in water or other liquids – agarose consists of long sugar molecules and gelatin consists of a protein called collagen (see Lesson Plan on Building a Molecular Model of Jello). Adding sugar to the gel increases its viscosity. Diffusion times are slower in a viscous gel containing 20% sugar, than in a gel without sugar. Jello contains 1.6% gelatin and 15% sugar – dyes will diffuse more slowly in Jello plates than in 1.6% gelatin plates without sugar. Diffusion times are also slower in viscous liquids, as well as viscous gels, compared to water. The cytoplasm of cells is very viscous – the viscosity of the cytoplasm is thought to be similar to glycerol or molasses. Large particles in the cytoplasm, such as ribosomes or mitochondria, require very long times to diffuse long distances (see Howard, 2001 Example 4.3).

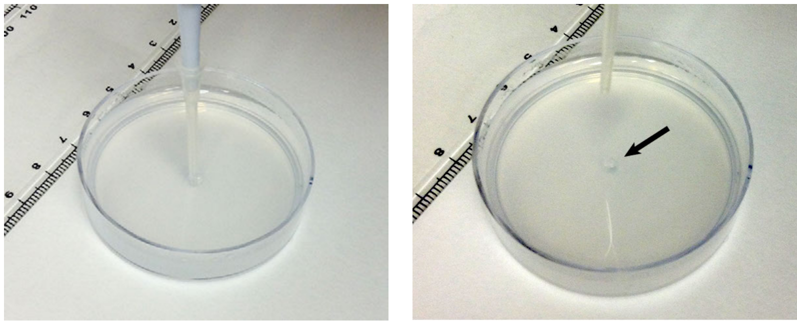
5. **Advanced topic 1**: diffusion times increase with distance as the distance squared. This means that particles diffuse short distances rapidly, but long distances require much more time. The time required to diffuse a given distance is the distance squared – this means that if it takes 2 hours to diffuse 1 *cm*, to diffuse twice the distance will require 2 *hr* x (2 *cm*) squared = 8 *hr*, or 4x the time that it took to diffuse 1 *cm* (see Howard, 2001).

Diffusion in two dimensions, e.g., within the cell membrane, is given by $x^2=4Dt$, where x is the displacement in *cm*, D is the diffusion coefficient in cm^2/s, and t is time in seconds (Berg, 1983). The relationship between diffusion distance and time can be demonstrated by measuring the distance diffused by the dye molecules in the dye spot, or the radius of the spot at each time point, squaring the radius, and plotting the radius-squared versus the diffusion time (**Figure 5**). The points can be fit to a line – this demonstrates that the diffusion time is linearly related to the diffusion distance, or radius, squared. The slope of the line is equal to $4D$. Dividing by 4 will give the value for D, the diffusion coefficient, for the dye in 1% agarose, 3% gelatin or Jello (as shown).

6. **Advanced topic 2**: the diffusion coefficient D is a property of molecules or particles in solution. It can be determined by the Stokes-Einstein equation, $D=kT/6\pi R_S\eta$, where k is the Boltzmann constant, 1.381×10^{-23} J/K, T is absolute temperature in K, R_S is the Stokes' radius (or hydrodynamic radius) of the molecule or particle, and η is the viscosity of the liquid. If the Stokes' radius of a molecule or particle and the liquid viscosity are known, D can be calculated from the Stokes-Einstein equation.

Figures

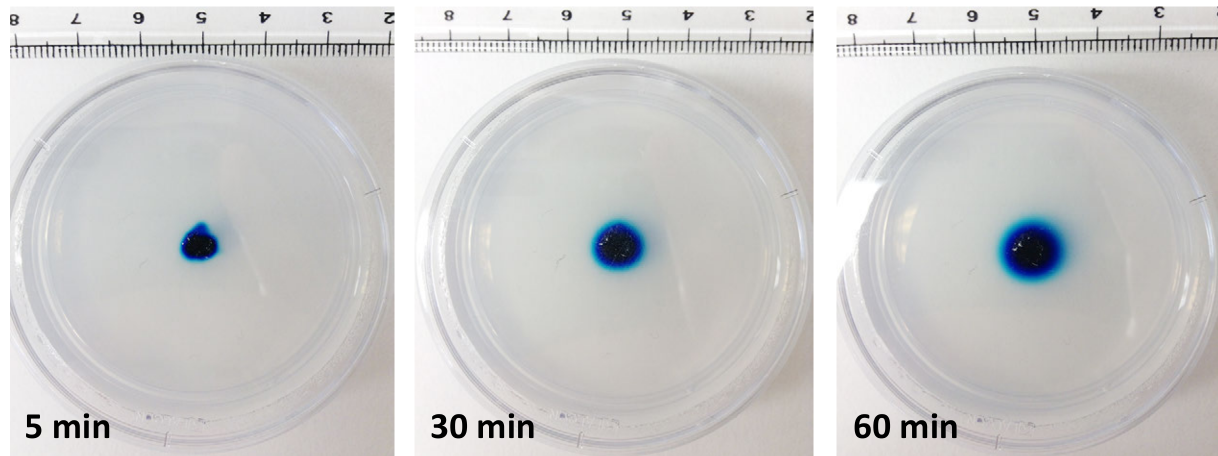
Figure 1 Making a Hole in the Agarose



Left, using a cut-off pipette tip (as shown) or a Pasteur pipette, make a small hole in the agarose for the dye. A round wooden toothpick can be used to do this instead. The hole should be positioned far enough from the edge of the plate so the diffusion of the dye spot can easily be observed.

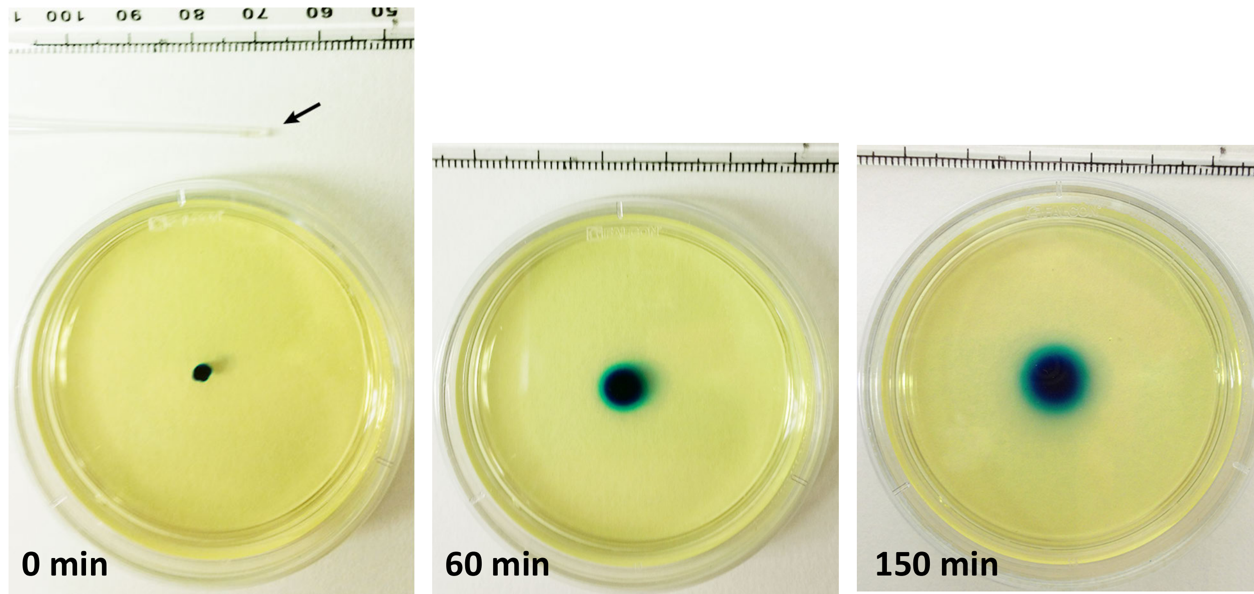
Right, the arrow points to the hole in the agarose.

Figure 2 Diffusion of Methylene Blue in Agarose



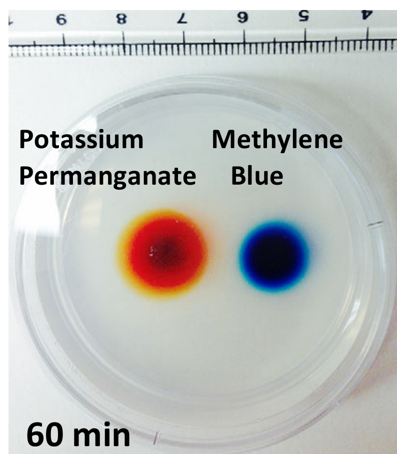
Methylene Blue dye was added to the hole in the 1% agarose gel. The photos were taken approximately 5, 30 and 60 minutes afterwards. The increase in size of the blue dye spot is caused by diffusion of the dye in the agarose.

Figure 3 Diffusion of Methylene Blue in Jello



A small hole was made in a Jello plate using a glass Pasteur pipette (arrow), as shown in **Figure 1**. Methylene Blue dye was added to the hole and the photos were taken 0, 60 and 150 minutes later. As the blue dye diffuses in the Jello, the dye spot increases in size.

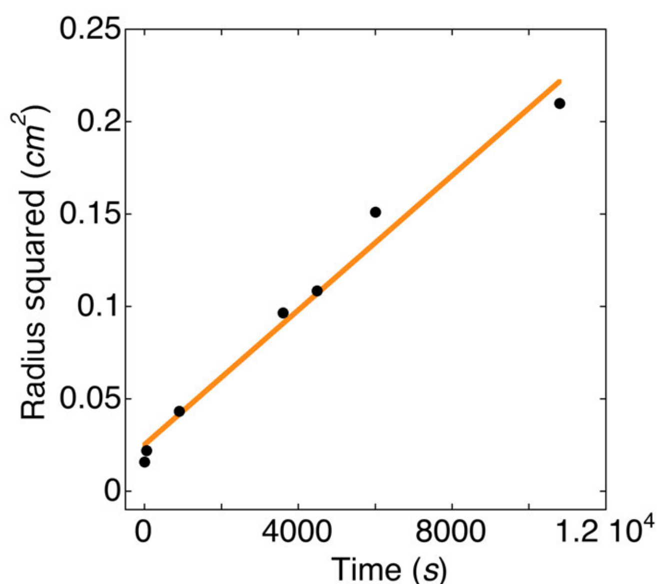
Figure 4 Diffusion of Two Dyes in Agarose



Potassium Permanganate (left, orange) and Methylene Blue (right, blue) after diffusion for 60 minutes in 1% agarose. Potassium Permanganate is approximately half the molecular weight of Methylene Blue and diffuses more rapidly than Methylene Blue, resulting a slightly larger radius for the Potassium Permanganate dye spot than for Methylene Blue.

Note that Potassium Permanganate forms aggregates in the dye spot and diffusion gradually becomes slower than Methylene Blue; after ~3-4 hours, the Methylene Blue dye spot will be larger than the Potassium Permanganate dye spot.

Figure 5 Plot of Diffusion Distance vs Time for Methylene Blue in Jello



Methylene Blue diffusion in Jello (see images in **Figure 3)**. The diffusion distance, which is equal to the radius of the dye spot, was measured at different times, squared, and plotted against time after converting time from minutes to seconds. The points form a line, showing that the diffusion distance (or radius) squared increases linearly with time. Given $x^2=4Dt$ for 2D diffusion, the slope of the line fit to the points is $4D$. After dividing the slope by 4, the value of D for Methylene Blue diffusion in Jello was found to be ~4.55×10^{-6} cm^2/s or ~455 $\mu m^2/s$ in this experiment. Note that inaccuracies in measuring the dye spot size, as well as differences in gel plates and room temperature may cause your value for D to differ from ours.

References

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