BEER'S LAW SIMULATION

In a guided-inquiry format, this lab uses a simplified example of what is happening in the cuvette of a spectrophotometer to allow you to provide you a molecular understanding of the (a) fundamental process of absorption and (b) mathematical derivation of Beer's Law.

BACKGROUND

Beer's Law (also known as the Beer-Lambert Law) describes the relationship between the amount of light absorbed by a solution and its concentration. The *absorbance* (A) of a solution of concentration c in a cuvette with path length b is given by

$$A = -\log\left({}^{I}/_{I_0}\right) = \varepsilon bc,$$

where I_0 is the intensity of light entering the solution, I *is the intensity of the light exiting the solution, and* ε is a constant called *molar absorptivity*, which is unique to each solute/solvent system. By now, you have probably held a cuvette and used it to measure the concentration of a solution using the principle of Beer's law. But can you explain exactly what is going on in the cuvette at the molecular level? To help you visualize the process of absorption as well as provide a quantitative basis of what is occurring in the cuvette, you will be interacting with a simulation that was hand-crafted here at UW-Madison.

The applet used in this lab simulates light going through a cuvette (*i.e.,* a sample cell) by dropping small spheres ("photons") through a box filled with cylinders ("solute particles") arranged in a discrete number of layers. When a photon touches the surface of a cylinder, it gets stuck, emulating the absorption of light by a solute. The photons are arranged randomly along a single (x-y) plane and can move uniformly through the sample cell (z-direction). After "crossing" each layer, their positions are always re-randomized along the x-y plane. The photons that are not captured by any cylinders make it through (are transmitted through) the cuvette and are detected by the screen on the bottom (the detector).

On screen, you will see a few buttons (at the top), values you can edit (on the bottom), and a running log about the data obtained from the simulation (very bottom). Every time you reload the simulation/web page, this box will be cleared.

The following controls will be useful:

- Run/Pause, which starts or pauses the simulation
- Reset Photons, which places the photons back at the "source," allowing you to repeat the experiment. No need to click "Run" again if the simulation is already running.
- Reset View, which resets the original view of the box (in case you zoomed in too much and got lost.)
- Update Cylinders, which applies any changes you made to the number of layers or number/radius of cylinders and rearranges them in the box.
- Grid on/off: Enables/disables a grid visualizing the box. Each square in the grid is 1 centimeter.
- You can also rotate the view of the cylinders by right-clicking and dragging anywhere inside the image.

It is recommended that, before starting the lab, you play around with the controls. See what values are given to you; try to resize the simulation. For good measure, see happens when you don't click Update Cylinders. If you understand the simulation controls, it will make it MUCH more efficient for you when collecting data from the simulation. You can do this as part of your pre-laboratory preparation!

Whenever we are collecting data in general, we have always wanted to collect replicates to understand how random error can impact a measurement. Thus, when assessing each set of conditions, make sure you perform at least 5 trials with 1000 photons each (7-8 trials is recommended). Also, as you know from learning the scientific process, there are logical steps for you to follow when trying to analyze a problem. Thus, for each of the questions below, using your lab notebook, we recommend the following, already familiar steps:

- Create data tables to collect/log data (*e.g.,* Figure 1)
- Collect data
- Perform calculations
- Critically evaluate the answer (*e.g.,* Does the result make physical sense?)
- Phrase the answer in a sentence

	Radius:		Radius:		Radius:	
Trial #	absorbed		absorbed		absorbed	
1						
2						
3						
4						
5						
6						
7						
8						
Avg.						
St.Dev.						

Figure 1. Example data collection table for experiments run with the simulation.

BEFORE YOU TAKE THE QUIZ

- Watch all associated video content to introduce you to the simulation.
- Understand the basics of absorption spectroscopy, including the instrumental components, such as the light source, the cuvette, the sample, and the detector.
- Recognize Beer's Law and be able to define what each variable means.
- Be able to convert between transmittance, percent transmittance, and absorbance.

PRELABORATORY EXERCISES

1. Briefly open the Beer's Law simulation URL (bit.ly/BeerLawSim, case-sensitive!) and take a look at the cylinder illustration and controls. This simulation serves as a model you can use to explain the Beer-Lambert relationship between light intensity (I/I_0), concentration (c), molar absorptivity (ε), and path length (b). Identify and write down in your lab notebook how you think the quantities I, I_0, c, ε, and b are represented in the simulation (that is, which properties of the spheres, cylinders, and box relate to each quantity).

EXPERIMENTAL

Open the Beer's Law Simulation URL. Use the simulation to perform the virtual "experimental trials" listed below, remembering to record your observations, calculations, and predictions in your notebook when prompted (usually, by underlined text). While the post-lab questions are at the end (*RESULTS/CALCULATIONS*), you are welcome to answer and work through post-lab questions as you go along in the lab.

1. For 1 layer with 2 cylinders, record the photons absorbed for *at least three* different values of the cylinder radius with 1000 incident photons for each radius. Make sure you conduct enough trials. See an example data table in Figure 1. In the shaded areas of the table, you may choose another variable that you want to measure or keep track of (*e.g.,* cylinder cross-sectional area, % transmitted, *etc.*).

> *In your lab notebook*: Describe any relationship you observe between the radius of the cylinders and the number of absorbed or transmitted photons.

2. *What do you predict the transmitted number of photons to be for 3 cylinders of radius 1.8 cm on one layer? Explain the reasoning for your prediction.*
   Test your prediction by performing another set of trials.
3. For the same number and radius of cylinders per layer (3 cylinders, 1.8 cm), *what do you predict the number of transmitted photons to be when you add a second layer for the photons to pass through? How about when a third layer is added? HINT: Remember that the photons captured by the first layer do not continue to the next layer!* Test your hypothesis by performing another two sets of trials.

> If you find it difficult to identify a relationship with just your current sets of trials, or if your prediction was significantly off, feel free to select other radii or numbers of cylinders and run more trials until you feel comfortable you understand how the arrangement and size of cylinders affects the photons transmitted through a layer.

4. Run a set of trials by setting the number of layers to 5, choosing a fixed cylinder radius > 1.5 cm, and varying the number of cylinders per layer. Remember to conduct enough trials and to make a table!
5. If you make a plot with the number of cylinders per layer on the x-axis, which dependent variable do you need to plot on the y-axis to obtain a *linear, positive* relationship according to Beer's Law? Obtain 4 data points and test the linearity of your plot. *HINT: the y-variable should NOT just be the number of photons absorbed!*

RESULTS/CALCULATIONS

Fill out the answer sheet for this experiment completely. Create a single PDF of your answer sheet and images of your notebook pages and submit to Canvas. Answer the following post-lab questions as precisely as you can in your lab notebook:

1. From the data collected in your Experimental step 1, determine a mathematical relationship that you can use to calculate the expected fraction of photons absorbed by

one layer, given the number and radius of the cylinders as well as the total layer area. Explain why this relationship holds. HINT: What is the probability that a randomly positioned photon will get captured by a cylinder?

2. How does your prediction from Experimental Step 2 compare with the result obtained? Using your result from question 1, explain why your prediction does or does not agree with the results. How do your predictions from Experimental Step 3 compare with the results obtained? Again, using the mathematical relationships you have discovered so far, explain why your prediction does or does not agree with the results.Using the mathematical relationship(s) you have determined, calculate how many transmitted photons one would expect to get in Experimental Step 3 (3 cylinders/layer, 1.8 cm) if (i) a fourth or (ii) fifth layer was added (you don't need to test this with the simulation). Sketch a plot of the expected number transmitted photons against the number of layers. How would you describe the shape of that plot?

3. Actual solutions have a continuous (rather than discrete) arrangement of molecules across their length. Let's now put things in mathematical terms, to see how your above discoveries about cylinders neatly arranged in discrete layers can apply to a continuous situation. Consider the following variables:

   - N = number of cylinders *per cm³* (not per layer!)
   - σ *(sigma)*= cross-sectional area of each cylinder in cm^2
   - A = *cross-sectional area of each layer*
   - h = height of one layer
   - b = total height of box
   - I = *number of photons transmitted*
   - I_0 = *total number of photons*

   Using the above variables:

   i. Express the *volume* of each layer.
   ii. Express the number of cylinders per layer.
   iii. Express the total cross-sectional area occupied by all cylinders in one layer.

4. Based on your observations in Experimental steps 1-3 and Post-lab Question 5, what is the *fractional* change in number of transmitted photons ($\Delta I/I$) passing through a single layer of thickness h? (*HINT: It will be negative since photons are 'lost' via absorption.*)

5. Now consider your answer to Post-lab Question 6 for an infinitesimally small change in number photons dI when passing a very thin layer of thickness dh. To find the number of photons transmitted through the entire sample (thickness b, initial number of photons I_0), integrate both sides of that expression with respect to dI and dh. (*HINT: This integration should give you Beer's law!*)

   - Math Help #1: $\int_{x=a}^{b} k\,dx = k(b-a)$
   - Math Help #2: $\int_{x=a}^{b} \frac{1}{x}dx = \ln(b) - \ln(a)$
   - Math Help #3: $\log_a(x) = \frac{\log_b(x)}{\log_b(a)}$
   - Thus, Math Help #4: $\log_{10}(x) = \frac{\ln(x)}{\ln(10)}$

6. From Experimental step 5, comment on the linearity of your collected data.
7. How would your plot in Experimental step 5 change if the cylinder radius was increased? What property would this change be analogous to in a solution following Beer's law?

CHALLENGE QUESTIONS FOR YOUR LAB NOTEBOOK

Provide answers to the best of your ability to the following questions. Freely share your ideas and predictions. Any honest attempt to answer the questions will be rewarded full points!

1. After completing this activity, have your answers the Pre-Lab Question 1 changed? If so, revise your answer here. If not, write "See Pre-Lab Question."
2. Discuss to what extent the variables of Beer's Law (intensity, concentration, absorptivity, and path length) are accurately represented in the simulation. Specifically consider the context of an absorption measurement. Can you propose any improvements to how the quantities are represented?
3. Do you have any additional improvements to propose for the simulation?