Objectives
This experiment involves the interaction of light with molecules that produce color, and some ways we can use light to determine the concentration of colored substances.

There are three parts to this experiment. In the first part we will examine the absorption of light by colored substances, and learn to predict the colors that result when white light passes through two solutions of different colors. We all know that blue and yellow make green. Here is why.

The second part of the experiment involves using selective absorption of light to determine the concentration of a colored substance. To accomplish this, we will measure this sample's absorption of a specific color of light and compare it with a set of standards.

The third part of this experiment will involve fluorescence. Tonic water contains quinine, which will fluoresce when struck by 400 nm (violet) light. We will use this phenomenon to compare the amount of quinine in two different brands of tonic water.

Background
Color is a natural phenomenon that many of us take for granted. We are concerned about the color of our clothing or the color of our cars, but we seldom stop and think about the many uses of color. Traffic lights and signs depend very much on color to communicate important messages to people. Scientists also use color to obtain information about chemical compounds. The area of science that concerns reading the light absorbed or reflected by a substance is called spectroscopy. The subset of spectroscopy that involves visible light is called colorimetry. You have already used emission spectroscopy to find the identity of unknown elements. Finding the identity of a substance is a qualitative experiment. Finding the amount is a quantitative experiment. One of the parts of this experiment will involve using light to find out the concentration of an unknown sample. To begin, let's discuss what causes the colors that we see.
Figure 1. A stop sign appears red because it absorbs violet, blue, green, and yellow wavelengths of light. Only the red and orange are reflected back to the observer.

Color Formation, or “Why is the Sky Blue?”

Many people believe that colors are emitted by colored substances. In fact, the opposite is true. White light is a combination of all the visible colors, red through violet. The paper on this page appears white because it reflects all of the visible colors and absorbs none. The ink on this page is black because it absorbs all colors and reflects none. The paint on a stop sign is red because it absorbs all the colors in white light except red (Figure 1). The same principle holds for the sky. Some of the light from the sun becomes scattered by the Earth’s atmosphere. During this scattering process, air molecules absorb all colors except blue.

We see color because materials all around us absorb some of the colors that make up white light. What happens during this absorption? Where do the colors “go?”

You have learned that light is made up of extremely small packets of energy called photons. Photons interact with the electrons that surround atoms, causing these electrons to “jump” to a higher energy level. If the energy of the photon matches the difference between two energy levels, the photon will transfer its energy to the electron. This extra energy causes the molecule that absorbed the photon to rotate or vibrate more vigorously until the excess energy dissipates. We feel these molecular vibrations as heat. That is why dark objects (ones that absorb a lot of light) become hot when they are placed directly in sunlight.

Safety Precautions

- All general laboratory safety rules apply to this experiment, including wearing eye protection.
- Potassium permanganate solution is poisonous and will stain your hands or clothes.
- Be careful not to jam vials in the spectrophotometer sample holder.
Materials

Equipment
- MicroLAB FS-522, film canister light shield
- MicroLAB Visual Spectrophotometer module
- White light source

Supplies
- Empty spectrophotometer vials

Reagents
- Spectrophotometer vials containing solutions of blue, green, yellow, and red food color, and of potassium permanganate solution prepared by your laboratory instructor
- Tonic water (quinine) standards and unknown

Experimental Procedures

Part I. Observing Molecular Absorption Spectra

It is conventional wisdom that color is something that an object gives off. My shirt is blue because it gives off blue light. Trees are green because they give off green light. This is not how it works. White light contains all of the colors of the visible spectrum—violet, blue, green, yellow, and red. If all of these colors are present, our eyes tell us that we are seeing “white.” If some of the colors are missing, we see color (a different color) instead of white.

To explore this phenomenon we are going to use a special kind of spectroscope that can hold a vial of colored solution in a manner that allows you to observe two simultaneous spectra—one of light coming through the vial, and one of light shining just under the vial.

Figure 2. This spectroscope will help you observe the absorption spectrum of colored liquids, as well as the emission spectra of hot gases. One looks in the diffraction grating at the left end, through the vial, and on toward a white light source. The vial is suspended half way into the light path. You observe the absorption spectrum of the sample with a reference white light spectrum below it.
We will start out with a few observations with the spectroscope. Make sure both you and your lab partner see all of the spectra that we will discuss. We are at somewhat of a disadvantage in this lab manual, because we will be showing pictures in black and white, and you will be seeing them in color in your spectroscope. We will indicate the colors with notes in the following figures, and you can look at these pages in color on your course web site.

To look at colors with a spectroscope, we have to have “full spectrum” white light. Although fluorescent light fixtures are advertised to do this, only incandescent lamps or sunlight reflected from a cloud really do a good job for this experiment. Use your spectroscope to look at the overhead lamps in your lab. They are probably “full spectrum” fluorescent lamps, but they fool your eye into thinking they are white by just sending blue, green, and red. Your lab instructor will provide “full spectrum” white light either with halogen desk lamps around the lab, or with an overhead projector shining on a screen. If you use desk lamps, shine them on a piece of white paper and then view the illuminated paper through the spectroscope. This gives a good even illumination. (Figure 3.)

Figure 3. An ordinary desk lamp with a halogen bulb makes a good light source for this experiment. The white sheet of paper diffuses the light and gives a smooth background for your absorption spectra observations.

Without any vial in the spectroscope, view the white spectrum on your screen or paper. Your spectroscope should be pointed at the paper. You will see a slit in the center, and on either side to left or right a spectrum that starts at violet toward the center and moves through the spectrum to red. You can select either the right or left spectrum—they will both behave the same. Make sure you and your lab partner both can see the “white” spectrum (Figure 4). This is an interference pattern of light waves like that discussed in the previous experiment. Short waves reinforce close to the center—long waves farther from the center. Violet light waves are shorter than red light waves.
Figure 4. When you look straight into the spectroscope, you can see a spectrum on each side of the slit. Violet is toward the center, red on the outside. Violet light waves are shorter than red light waves.

Now place a “blank” vial with water in it into the sample holder. Look in the front of the spectroscope. The vial only sits half way down into the light path. Half of the light will go through the vial and half below it.

Look again at your white light source. You will see two spectra separated by a black line. This black line is caused by the bottom of the vial. (Figure 5.)

Figure 5. When a vial is inserted into the sample holder, the bottom of the vial sits half way down the slit and forms a line between the top “sample” spectrum and the bottom “reference” spectrum.

Now pick a purple sample—a dilute solution of potassium permanganate works well. Your lab instructor will have several vials of colored solutions for you to work with. All of them will be food color except for the potassium permanganate. Don’t get KMnO₄ on your hands or clothes. It will stain them.

Look at your white light source with the purple solution in the vial holder. You should see a spectrum like that illustrated here in Figure 6. In this figure we cropped the photo to show only the right-hand spectrum visible in your spectroscope. You can see just this spectrum by looking to the right in the diffraction grating.
The purple solution is not giving off purple light. It is absorbing green light. What is left over after going through the sample is violet and blue and yellow-orange-red. This combination of violet–blue–(green missing)–yellow–orange–red is translated by your eye as “purple.”

Try this with blue food color. It isn’t giving off blue light. The blue solution is absorbing a chunk out of the yellow-orange-red part of the visible spectrum. This combination of violet-blue-green and then long wavelength red is translated by your eye as “blue” (Figure 7).

Now try the yellow sample. Shade in the spectra drawings to the right in Figure 8 to indicate the location of the transmission and absorption bands for yellow. What colors does your eye translate into “yellow”? 

Figure 6. The purple solution (top spectrum) absorbs green light. The reference spectrum below shows the whole visible spectrum.

Figure 7. The blue solution absorbs yellow-orange-red light. Some long-wavelength red gets through.
Figure 8. Shade in the colors absorbed by a yellow sample. Draw the arrows below to show which colors are transmitted by the sample.

Try this again with a green sample. Shade in the spectra drawings to the right in Figure 9 to indicate the location of the absorption bands for green. What colors does your eye translate into “green”?

Figure 9. Shade in the colors absorbed by a green sample. Draw the arrows below to indicate which colors are transmitted by the sample.

Part II. Measuring Molecular Absorption Spectra with the MicroLab FASTspec Scanning Spectrophotometer

The MicroLAB FASTspec scanning spectrophotometer makes measurements at sixteen different wavelengths in the range 360–940 nm. It generates these measurements with sixteen carefully spaced Light-emitting-diodes arranged concentrically around the sample (Figure 10). The LED arrangement is such that each LED drives a transmitted light sensor, a scatter sensor located at 90 degrees to the light beam, and a backscatter sensor located at 135 degrees from the direction of the beam. These three sensors permit simultaneous measurement of transmission and absorbance of light through the material in the sample vial, and measurement of light scattered by particulates in the sample (turbidity), or generated by fluorescence. The backscatter sensor extends the turbidity range of the instrument by about a factor of three.
Running a Blank
The first step in a spectrophotometric measurement with the FASTspec scanning spectrophotometer is to run a “blank.” This is a distilled water sample that by definition will transmit 100% of the light at every wavelength in the spectrophotometer’s range. It is quite easy to run a blank:

- Turn on the MicroLAB FS-522 and start the spectrophotometer program. (Figure 11.)
- Put a distilled water blank in the sample holder, and install the light shield.
- Click the “Read Blank” button. Each of the LEDs will be set at approximately the same light level. After all sixteen have been set up, the program normalizes the data to the lowest LED intensity. After your blank has been set up, remove the light shield and blank, and run a sample with nothing in the sample chamber. Just enter “test” for the name of the sample, and leave the concentration at zero. Hit “OK” to read.
Look down in the sample vial and hit “OK.” You will see the FASTspec spectrophotometer make two scans, each taking about one second. The first scan reads transmitted light for each wavelength, and the second scan reads scattered light at right angles to the light beam. You can click “Remove” to remove this test spectrum.

**Spectral profiles and the difference between transmission and absorbance.**

Now put the purple sample in the sample holder, replace the light shield, and click “Add a sample.” Name the sample “purple.” To run a spectral profile, you don’t have to enter a concentration. We will do this later in the Beer’s Law experiment. Click OK, and in about two seconds you will have a % Transmission spectral profile for this sample.

Compare the MicroLAB spectral profile with the visual profile you saw with the spectroscope.

Look at the menu bar at the top of the MicroLAB spectral profile. The default display is percent transmission. Click on the “Absorbance” button. Absorbance is the inverse of transmission—more properly stated in chemist’s terms, it is the log of the inverse of the transmission. Chemists like to use absorbance because it focuses on the important thing about spectra—what colors are absorbed. Absorbance also increases linearly with concentration, which makes it a great tool for determining the concentration of unknown solutions.

You can flip back and forth between absorbance and transmission displays using the buttons on the top menu bar. You can also flip between a colored histogram display and a line spectral profile with a button in the upper chart. Decide which kind of spectral profile display you prefer to use. The figures in this part of the text use the histogram display.

Potassium permanganate is purple because it absorbs the green part of the white spectrum and leaves the violet blue and yellow-orange red. Look carefully at Figure 6—can you relate the transmission graph and the absorbance graph presented in Figure 13 to the visual picture you got through the visual spectroscope?
Using spectral profiles to predict the result of color mixing.

Color mixing is an interesting phenomena, and one that can give you a chance to make sure you understand the principles of absorbance and transmission.

We will try three quick color mixing experiments.

Select three vials—your potassium permanganate sample, your blue sample, and your yellow sample.

Line up the purple potassium permanganate and the blue samples so you can look through them. Position them so that you can see the purple, the blue, and the “mix” where you look through both vials at a white light (Figure 14). What color do you see?
Start the FASTspec Color Comparison program. You will have to run the blank again—this blank display may not include the graphic display as the several wavelengths are set to 100%. With this program you can run different colored samples, and then display the spectral profiles one above the other to compare them. You can also “mix” the two colors in software, to predict the transmission or absorbance of the combination of the two colors.

When you have run the purple and blue samples, click the “Mix/Compare” button, and check the box that says “Show Mixing Controls.” You will see a screen as shown in Figure 15.

You can see from about 400 nm in the violet to about 680 nm in the red. This area is shown in a light-colored box in Figure 15.

Which of the colors you can see will pass through both solutions? Violet and blue and a little green will go through both. Yellow and red will pass through the purple solution, but will not pass through the blue. All you will see is violet. Does this match your observation when looking through the purple and blue solutions?

Now click the absorbance button on the top menu bar to view the absorbance of the two samples. Also click the “MIX” button. The third graph will show the effect of electronically adding the absorbances of the two top solutions (Figure 16). As above, the visible region is highlighted. The region of lowest combined absorbance is in the violet-blue. Because absorbance is a logarithmic relationship, and your eye reacts logarithmically to changes in light intensity, the absorbance “mix” gives the best estimate of what you will see when you “mix” or look through the two colored vials together.
Try running the slider up and down to predict the effect of changing the relative concentrations of the purple and blue solutions.

![Figure 16](image)

*Figure 16. When you “mix” or pass light successively through two colored samples, the absorbances for each wavelength add. It is easy to look at the combined graph (bottom) to see where the absorbance will be least (small box), and which colors will come through the “mix” most easily. In this case it will be violet-blue light.*

The two spectra shown in Figure 17 show the effect of “mixing” purple (top spectra) and blue (bottom spectra). The only area of common transmission is in the violet, with a very small component of long-wavelength red.

Here are two additional experiments for you to try:

- Try looking through the purple and yellow vials. What color do you see? What can you predict with the color comparison program?
- Try the same experiment with blue and yellow vials. What color do you see? What can you predict with the color comparison program?

![Figure 17](image)

*Figure 17. The purple solution (top spectrum) absorbs green light. The blue spectrum below absorbs green and most of the red. What is left passing through both is violet, with a little transmission in the far red.*
Part III. Percent Transmittance, Absorbance, and Concentration

How can scientists use this selective absorption of photons to find solution concentrations? Figure 1 showed how light is absorbed when it reflects off of a surface. Certain colors of light may also be absorbed when shown through a translucent substance, such as a solution. In Figure 18 a white source light shines through a solution of green food coloring. Molecules in the solution absorb yellow and orange wavelengths of the source light, making it appear green after it passes through the solution. If a light sensor is placed on the opposite side from the light source, the sensor could measure how much light was cut out by the solution. The more concentrated the solution, the more light would be absorbed.

Figure 18. Light passing through green food coloring.

Scientists describe the interaction of a solution with light in terms of percent transmittance. Percent transmittance refers to how much of a certain color of light gets through a solution. Distilled water has 100% transmittance, while an opaque block has 0% transmittance. The light sensor you will use in this experiment contains a semiconductor that produces electrical current based on how many photons strike its detector. Because of this fact, the light sensor measures percent transmittance directly. If more photons pass through the solution and strike the light sensor, more electrical current flows.

Suppose we have three identical vials of a substance that each have 50% transmittance of the source light. In Figure 19a, one vial is placed in front of the light, and half of the source light shines through the vial. A second vial is placed next to the first in Figure 19b, and 50% of the remaining light is cut out, making the total percent transmittance of two vials placed side by side 25% (not 0% as you might expect). In Figure 19c, a third vial reduces the remaining light by half, and now 12.5% of the original light is being transmitted.
Figure 19. The effect of placing 50% transmitting vials in front of a light source. Each vial absorbs half of the light that strikes it. The result is a curve (Figure 20) relating concentration to amount of light passing through the system.

A table of percent transmittance for 0–4 of vials, as well as a graph of this data, is shown in Figure 20. The raw data does not make a straight line. It would be very difficult to fit this data to a linear equation and then predict what the percent transmittance would be for a solution of unknown concentration. We need to perform a curve fit to find the function that allows us to express percent transmittance as a straight-line function.

By taking the logarithm of percent transmittance and multiplying this value by –1, we find a linear relationship between light and the amount of absorbing substance in the light path. Scientists have given the name absorbance to the quantity $-1 \times \log$ (percent transmittance). Figure 21 shows what the data in Figure 20 looks like when percent transmittance is converted to absorbance.
Percent transmittance is easier to measure, but absorbance is directly related to a solution’s concentration by a relationship known as Beer’s law. In this experiment you will measure percent transmittance, convert that quantity to absorbance, and then use absorbance to find the concentration of your unknown solution.

In equation form, Beer’s law is:

\[ A = \varepsilon \cdot \ell \cdot c \]

- \( A \) is the solution’s absorbance (usually converted from percent transmittance).
- \( \varepsilon \) is the solution’s molar absorptivity (a property that is different for different solutions and different light colors).
- \( \ell \) is the cell path length, or the distance through the solution that the light travels.
- \( c \) is the solution’s concentration.
Experimental
In this part of the experiment we will check to see if the theoretical model relating solution concentration to absorbance works.

Prepare a blank and five different concentrations of a food color sample provided by your lab instructor.

Choose concentration as your x-axis variable.

Run a blank with your spectrophotometry program, and then successively measure the absorption spectrum of each of your five standards.

<table>
<thead>
<tr>
<th>mL Water</th>
<th>mL Food Color</th>
<th>Concentration Parts/10 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>2</td>
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<tr>
<td>12</td>
<td>8</td>
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<td>6</td>
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<tr>
<td>4</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Concept 1: There is a relationship between the amount of light absorbed and the concentration of the absorbing species in the solution.

Enter your samples by placing each vial in turn into the sample chamber and pressing “Add” until all of the samples have been entered.

After all of your samples have been entered, move to curve fit and try to fit the Transmittance curve. It will probably take a second order polynomial (quadratic) equation to fit this line. Transmission decreases with increasing concentration (Figure 22).

Figure 22. Transmittance decreases with increasing solution concentration. However, this is not a linear fit.
Now click on “Log Transmittance” (Figure 23). Make sure the autoscale function (lower right) is on. This line is straight, but decreases in value with increasing concentration.

![Log Transmittance graph]

**Figure 23.** The relationship between Log (T) and concentration is linear, but the value decreases with increasing concentration. Is there a better way to display this data?

Finally, Click on “Absorbance [-LogT]” (Figure 24). Absorbance increases linearly with increasing concentration. There is a direct (linear) relationship between the absorbance of light at a selected wavelength and the concentration of the absorbing species in the solution. This relationship is called “Beer’s law.”

![Absorbance graph]

**Figure 24.** The absorbance (-LogT) relationship is linear with increasing concentration. This relationship is called “Beer’s law.”
Concept 2: The slope of the Beer’s law Absorbance/concentration plot changes with wavelength. When it is greatest, the spectrophotometric method is operating at highest sensitivity.

With the plot set at absorbance, try selecting a different wavelength for your measurement. Click on the selected histogram. In Figure 25, below, we clicked on the wavelength of greatest absorbance for this sample, 502 nm. Note that the maximum absorbance was about 1.106 absorbance units, as compared to about 0.32 absorbance units for the 430 nm data shown in Figure 24.

![Figure 25. The slope of the absorbance/concentration graph is greatest when measured at the wavelength of greatest absorption by this sample.](image)

Press the button in the lower right-hand corner of the graph to turn the auto scale off. Now try selecting several different wavelengths to see what happens to the slope of the absorbance/concentration line (and the sensitivity of the analytical method) as the wavelength moves away from the wavelengths of principal absorption (Figures 26, 27).
Figure 26. Absorbance measured at 502 nm shows a slope of 1.106.

Figure 27. When measured at 470 nm, the slope of the line is 0.784. The analytical method is less sensitive when run at this wavelength than at 502 nm.

Concept 3: Molar Absorptivity Constant.
Beer's law states that the absorbance (A) of a sample at a selected wavelength is equal to the product of three factors:

- A constant called the molar absorptivity constant (a)
- The path length (b)
- The solution concentration (C)

\[ A = a \cdot b \cdot C \]

The slope of the absorbance/concentration line in this experiment is the path length times the molar absorptivity constant, for that compound at that wavelength.
When one moves away from the principal absorption wavelength, the slope of the absorbance/concentration line becomes less, and the measurement becomes less sensitive.

Try selecting a wavelength where this sample absorbs light very poorly –700 nm, for example. Turn autoscale back on, and look at your data. At this wavelength the sample hardly interacts with the light at all, and the result is simply noise (Figure 28).

**Figure 28. When a non-absorbing wavelength is chosen (700 nm in this example), the absorbance signal disappears into noise.**

**An Unknown Sample**

Switch to 502 nm. Now click on the fourth tab, “Read Unknown.” Place an unknown in your sample chamber (or click Add if you are doing a simulated experiment). MicroLAB’s software will calculate the concentration of the unknown and place an “X” on the absorbance concentration line (Figure 29).

**Figure 29. The concentration of an unknown may be determined by measuring its absorbance and mathematically spotting it on the absorbance/concentration line.**
What wavelength would you choose to get the most sensitivity for this particular colored compound?

**Concept 4: Deviations from Beer’s law.**

If the wavelength of light used in a spectrophotometric measurement does not match the absorption band of the sample well, less absorption will occur at high concentrations than would be predicted by Beer’s law. Figure 30 shows the same sample, but this time measured at 525 nm. Try this measurement, and try fitting the data with a straight line, a linear curve fit. Note that the points do not exactly fit the line, and that the correlation coefficient is 0.9993 for this data (Figure 30).

![Figure 30. When the analytical wavelength selected is removed from the wavelength of principal absorption, the Beer’s law plot deviates from a straight line.](image)

**Part IV. Fluorescence**

Some compounds will fluoresce when their molecules are struck by photons of ultraviolet light. Quinine is such a compound. Quinine has been known for a long time as an effective antimalarial drug. In colonial India, the British would take this drug as protection against the malaria mosquito. To make it palatable, they mixed it with tonic water and gin—hence the “gin and tonic.” Today tonic water still contains quinine.

Fluorescence is the molecular analog of emission spectrum of gases. Ultraviolet light promotes electrons to higher energy levels, and as the electrons fall back light is emitted. Fluorescence differs from emission spectra, however, because the rotation and vibration in molecules broadens each energy level, and one observes a band of emitted color instead of a sharp line as in gaseous emission spectra.

In this experiment, we will prepare a standard curve for Schweppes tonic water, and then compare the quinine content of full strength Schweppes with full strength Canada Dry tonic water.
The procedures are very similar to those for your food dye and chlorine experiments. Prepare a set of standard solutions, diluting Schweppes tonic water to make six standards with concentrations from 0 to 10 parts per 10 mL. (Use a dilution table such as is presented on page 96.)

Start the spectrophotometer program again, and run a new blank. However, instead of displaying absorbance, display fluorescence.

Each bar on the histogram shows the fluorescence produced by that color of light.

After you have run the blank, put the full strength tonic water in without a cap on the vial, cup your hand over the sample holder and look straight down into the sample. Then have your lab partner press “Add sample.” The MicroLAB spectrophotometer will scan all sixteen LEDs. When the ultraviolet LED comes on, you will be able to see a weak blue fluorescence shoot along the light path through the sample. Both you and your lab partner should look at this.

Then start the program over, run a new blank, and run your six samples, making sure to use a light shield. Use the histogram to pick the exciting wavelength that produces the most fluorescence, and then switch to curve fit to develop an equation for the line.

The amount of fluorescence a sample produces is directly related to the number of fluorescent molecules in the exciting light beam. So the graph of fluorescence and concentration should show a linear relationship.

Look at the fluorescence/concentration graph produced at three ultraviolet wavelengths, 360, 383, and 400 nm, by clicking on the appropriate histogram bar.

Which wavelength is most effective at producing fluorescence? Look at the fluorescence scale at the left of the histogram chart.

Which wavelength produces the best (most linear) calibration graph?

There is a subtle effect taking place here. If the sample absorbs light well at a wavelength also used to excite the fluorescence, the fluorescence may drop off as concentration is increased because the exciting photons can’t reach as many of the quinine molecules as you might expect.

Switch your display from fluorescence to absorbance. Which wavelength is absorbed best? Can you develop an explanation for why your calibration graph is not straight, and why it is better at some wavelengths than others?

After you have completed the curve fit, click on “Unknown,” and add a sample of Canada Dry tonic water. Use the MicroLAB graph to measure the concentration of the quinine in Canada Dry, as compared to the Schweppes.
Part II. Color Mixing

Draw the spectrum and identify the resulting observed color of the following mixtures.

a. Blue and Yellow

b. Purple and Yellow

c. Purple and Blue
Part III. Percent Transmittance and Absorbance

Using the following table, answer the questions:

<table>
<thead>
<tr>
<th>mL Water</th>
<th>mL Food Coloring</th>
<th>Concentration (parts/10 mL)</th>
</tr>
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<td>20</td>
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<tr>
<td>0</td>
<td>unknown</td>
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</tbody>
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1. Which mathematical relationship is most useful to relate concentration to the amount of light passing through the solution? % Transmission, Log T, or Absorbance.

2. How does the slope of the absorbance/concentration graph vary with wavelength? Is there an “optimum” wavelength for your colored sample?
## Part IV. Fluorescence

<table>
<thead>
<tr>
<th>mL Water</th>
<th>mL Stock Solution</th>
<th>mL Tonic Water</th>
<th>Concentration (parts/10 mL)</th>
</tr>
</thead>
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<tr>
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</table>

Compare the concentration of quinine in Schweppes and Canada Dry tonic water.

## Part V. Conclusion

Write a brief conclusion about today’s lab.