

How Do We See Colors? -- Lab #4

Is that Product Safe?

The Situation

You and your lab partners are serving as a team of interns at the pharmaceutical testing laboratories of the Food and Drug Administration (FDA). A claim has been filed against the Drugs-R-Us Company because some pediatricians believe that their "*No-Cough*" cough syrups do not contain as much medicine as is listed on the product label. Your job for the day is to devise a method to determine the actual concentration of the medicine in a sample of *No-Cough* and to be able to justify whether or not the medicine contains the correct concentration of the important ingredient.

According to the product labels, the following Drugs-R-Us product should contain the following amount of anti-cough medicine.

<i>Product Name</i>	<i>Medicinal Additive</i>	<i>Listed Concentration of ACM (mg/L)</i>
<i>Cherry No-Cough</i>	Red ACM	5.2

Your instructor will show you a sample of the product in question. The *No-Cough* syrups are clear fluids except for the addition of the anti-cough medicine (red ACM).

1. Carefully describe the appearance of this product. Since you are viewing it with optical devices (namely, your eyes), be sure to describe its optical properties based on your visual inspection.
2. How do you think the optical properties of the fluid would change if there were more ACM added?
 - Would it change the percentage of transmitted light? Explain.
 - Would it change the optical density of the sample? Explain.
3. Based on your inspection of the unknown and given known samples, what would you predict for the concentration of the unknown substance? Explain your reasoning for your prediction.
4. Suppose you had four different light sources: red, orange, green, and blue.
 - Which light source(s) would work best for studying the absorption of light for this sample? Explain your reasoning in detail.
 - Which light source(s) would be least effective for studying the absorption of each of the samples?

Task #0 Preparing the Colorimeter

The colorimeter is an instrument designed to measure the absorption of light at certain selected wavelengths. The colorimeter you will be using today contains 4 separate LEDs (light emitting diodes – a special kind of light source) to produce lights of four different colors. These colors are:

Color	Blue	Green	Orange	Red
Wavelength (nm)	460	565	630	697

In addition, the colorimeter has a photodiode to measure the amount of light that passes through a sample. The colorimeter you will be using today is interfaced to the computer, which will allow you to read the amount of transmitted light via the computer display.

- Recall from your previous work, what is the *percent transmitted*? How is it calculated and what do you need to know to be able to perform this calculation? What does it mean to have 0% transmission? What about 100% transmission?

Calibrating the Colorimeter

Before you can begin to collect meaningful data, you must calibrate the colorimeter. This means that you must assign meaning to the signals that are coming from the colorimeter and going to the computer. In order to assign meaning to the values, you will want to tell the computer what signal corresponds to 0 % transmission and what signal corresponds to 100 % transmission.

- Start up the computer, turn on the interface, and open the file "Lab#4 MBL – Colorimeter."
- The colorimeter's display should show: "Please calibrate."
- Fill a clean cuvette with clear water so that the water level is just below the bottom of the plastic cap. Cap the cuvette and dry it off with a paper towel. Try not to touch the two smooth sides of the cuvette. If you do, be sure to wipe off any fingerprints.
- To calibrate, press the following two buttons on the colorimeter at the same time:
"Select" button and "Start/Stop" button
- The colorimeter should show: "Insert reference then push SELECT."
- Place the water-filled cuvette into the colorimeter and close the cover. Make sure the smooth sides (without ridges) are lined up with the light path in the colorimeter. Ask your instructor for assistance if you're unsure about this step.
- Press the "Select" button. The colorimeter will now calibrate itself for the value of 100% transmission for each of the four available light sources.
- The colorimeter will show: "CAL done, push SELECT or START."
- You are now ready to calibrate the software....

Before you continue, you must have decided which color of light you will use first in your study of this substance. Make a note of your choice and reasoning for your choice in your logbook.

Calibrating the Software

- Leave the cuvette of plain fluid (no ACM additive) in the colorimeter.
- Select the color of light you will be using by pressing the "Select" button on the colorimeter until the name of the desired color of light is displayed.
- Double click on the colorimeter icon in the *ScienceWorkshop* window.
- Press the "Start" button on the colorimeter. The colorimeter is now "RUNning."
- When the reading stabilizes, click the "Take Read" button in the "High Value" row. You have now told the computer what reading should correspond to 100%.
- Press the "Stop" button on the colorimeter. The colorimeter is "STOPped."
- Click "ok" to leave the setup window on the computer.



The colorimeter and the *ScienceWorkshop* software are now calibrated. That means that you are ready to use that particular cuvette with that particular wavelength of light. In other words, you are ready to begin your experimental work.

Research Question

Can you find a way to relate the concentration of red ACM or blue ACM in a sample to its optical properties (such as percent transmitted and/or optical density)? If you can find a relation, then can you use it to predict the concentration of an unknown sample?

Task #1-1

Sample #1 with Light Color #1 (Sample color: _____ Light color: _____)

You will measure the percent transmitted for six different known concentrations of the ACM substance.

Take out the cuvette full of clear fluid and dump this fluid in the discard pail. Refill the cuvette with your first sample from a known concentration. Dry off the cuvette and securely put on the plastic cap.

Place this new sample into the colorimeter and press the "Start" button. You should also begin "MONitoring" with the *ScienceWorkshop* software. When the reading stabilizes, then record in your logbook the value of percent transmitted for this known concentration. Press the "Stop" button on the colorimeter to stop taking data.

Data Collection Hints:

- When taking data, always discard the old sample into the provided discard pail so that you do not contaminate the known solutions.
- In addition, to prevent contamination from sample to sample, be sure to "wash" the cuvette with the next sample to be analyzed. To do this, just fill the cuvette with the new sample, dump it out in the discard pail, and then refill it once again.
- Be sure to dry off the cuvette before placing it inside the colorimeter for each measurement.

Create a data table of the given ACM concentrations, measured percent transmission, and calculated optical density. Record data for six of the known samples for the color of light you chose to use.

Using graph paper and/or *Excel*, create a graph of **percent transmitted as a function of sample concentration** and another graph of **optical density as a function of sample concentration**. Include these graphs in your logbook.

6. Describe how the two optical properties changed as the concentration of the sample changed. Try to be as specific as possible including describing the shape of the best-fit curve representing each set of graphed data.
7. Is one of the graphs linear? If so, use the guidelines for creating a linear function model to determine an equation that represents the best-fit line to your linear data. Show your work for each step in creating this equation and be sure to write out the full form of the equation in your logbook.

You now have created a calibration curve of the optical properties for this specific sample and light source. You can use this information to estimate the concentration of ACM in the *No-Cough* syrup.

Obtain a sample of the *No-Cough* syrup from your instructor. Analyze it using the colorimeter. From your results and your model(s), predict the actual concentration of ACM in the *No-Cough* syrup. Explain your work and state your evidence for all conclusions.

Task #1-2

Sample #1 with Light Color #2 (Sample color: _____ Light color: _____)

You should now repeat your analysis of the six given samples, but using a different light source. Try picking one that should give you meaningful data.

8. State which color of light you will be using. Also, record a prediction as whether this light will be absorbed more or less than the light you used in the previous task.

Before you continue, do the following preparations:

- Once again place a cuvette of the clear fluid into the colorimeter. (Remember to "wash" it first.)
- Recalibrate the colorimeter by pressing the "Start" and "Select" buttons at the same time.
- Select the new color of light on the colorimeter.
- Recalibrate the software for this new color of light by again double clicking on the colorimeter icon in *ScienceWorkshop*. Be sure you are not monitoring the data when you do this.

Analyze the same known samples as you investigated previously using this new light color. Again, make a data table and include: known ACM concentrations, measured percent transmission, and calculated optical density.

Graph this new data in a way so that you expect to obtain a linear fit. Create a linear functional model representing this data.

Retest the unknown sample with this new light source. Based on the measured value and your model(s), predict the actual concentration of ACM in the *No-Cough* syrup. Explain your work and state your evidence for all conclusions.

9. Compare the models you obtained using the two different light sources. Can you explain why they are or are not different?
10. Compare the two experimentally predicted values for the unknown sample. Was one method better than the other? Why or why not? How do the values compare to your "guess" in question #3?
11. Based on your results, were the pediatricians correct in their concern that the *No-Cough* syrup does not contain the correct amount of the ACM additive? If you were a doctor or the parent of a child who was going to take the *No-Cough* syrup, would you be concerned? Why or why not?

Concluding Questions

12. In words, describe as specifically as possible the relation between percent transmission and concentration.
13. In words, describe as specifically as possible the relation between optical density and concentration.
14. According to *Beer's law*, the optical density (also known as the "absorbance") is proportional to the concentration of material in a sample solution for low concentrations. Do your data support this "law"? Explain why or why not.

Application Activity

As a forensic pathologist you happen to know that fresh human blood has an optical density (OD) of 0.30 and that as human blood is exposed to the air it gets darker and darker, at about the rate of an increase in its OD of about 0.10 per hour.

The police have been investigating a motor cycle accident that occurred at the intersection of 17th and South Street late last night. The blood from the unconscious victim was rushed to the crime lab in south Lincoln and was measured to have an optical transmission of about $9\% \pm 1\%$ at 8:00 am this morning.

Please help the police figure out when the accident is likely to have occurred. Be sure to take into account the uncertainty in the crime lab results. Explain your reasoning in language that both policemen and lawyers can understand.

Don't forget your concluding questions...

- *Accuracy statement*
- *Implications statement*