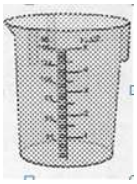
AP Lab Colorimetry-Light Path

Lab Equipment		Student Supplied:	
Two 12×75 mm test tubes[1] 	ruler 	blue food dye 	3×5 note cards or sturdy white paper 
 150 mL beaker	 plastic pipet	A sheet of white paper and a lamp or an LED display screen from a laptop, tablet, or Smartphone 	Five plastic cups. Don't use paper cups as they will leak over time. 
		transparent tape 	

Hazards: Blue food dye stains. Wash concentrated dye off surfaces and hands before it soaks in and you look like a Smurf.

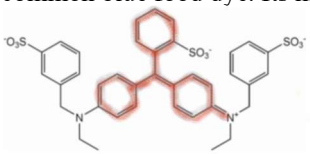
GOALS

1. To determine the relationship of the transmittance, concentration, and light path length
2. To develop a method of finding the concentration of a colored solution using absorbance of light.

Explanations

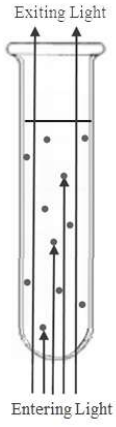
As electromagnetic radiation goes through a substance, specific wavelengths may be absorbed. The electron structure of some molecules can absorb certain visible wavelengths and the result of the subtraction of these colors is that the light beam no longer will appear to be white.

Blue dye #1, brilliant blue is a common blue food dye. Its molecular structure is



$C_{37}H_{34}N_2Na_2O_9S_3$
793 g/mol

The electrons in alternating single and double bonds, =C—C=C— C=, of dye molecule can absorb photons of light with wavelengths in the 630 nm range. The particulate drawing on the right shows 630 nm photons (red light) being absorbed by the molecules of blue dye #1. **This absorption of 630 nm red from the white light makes the solution look blue.** The number of molecules present will determine the amount of light that is absorbed.



[1] If you cannot get test tubes, you can use transparent flat-bottomed drinking glasses. They will work even better than test tubes. In effect you will be doing a giant experiment. Just increase everything by a factor of 10 so that you can fill the gasses the same way you would the test tubes.

Procedure:

Make up 5 solutions of known dilutions.

(1) Fill a150-mL plastic beaker with about 130 mL of water. Add 7 drops of food color to the water and stir with the measuring spoon to mix the solution. **You will want a dark solution that you can barely see though**. This will be your stock solution. As with most food dyes, blue dye #1 produces an intense color even in dilutions down to the millionth's molar. The concentration of dyes in food coloring varies depending on the brand of food dye used. However, you can set an arbitrary concentration for the stock solution and use it in calculating the relative concentrations of solutions. A reasonable estimate of the concentration for the stock solution of your food dye would be, 10. μ*M*.

(2) Label four plastic cups A through D.

(3) Using your graduate cylinder, add 20. mL of the stock solution to each cup.

(4) Add 10 mL of water to cup A, 20 mL of water to cup B, 30 mL of water to cup C, and 40 mL of water to cup D

(5) Assuming that the concentration of the stock solution is 10 μ*M* calculate the concentrations of the other four solutions.

Solution Cup	Total volume of solution	Concentration of Solutions $C_{stock} \times V_{stock} = C_X \times V_X$
Stock	20 mL	10 μ*M*
A	30 mL	
B	40 mL	
C	50 mL	
D	60 mL	

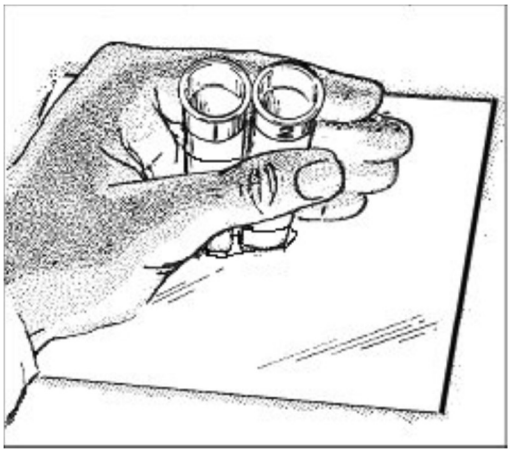
(6) Put together a double barrel test tube holder/light shield using paper. Take a 3×5 card and cut it to a height of 6.5 cm. Do the same with a second card. In the end, you should have two rectangles of white paper 6.5 cm in height by about 12.5 cm in length. Or you could take any stiff, white paper and cut two 6.5 cm × 12 cm rectangles.

(7) Roll one of the rectangles around the 7.5 cm test tube so that the top 1 cm of the test tube is showing. Tape the paper tube along its length so that it remains a cylinder when the test tube is removed. Repeat to make a second cylinder.

(8) Tape the two cylinders so that they are parallel to one another to make a double barrel test tube holder. Then secure a strip of transparent tape over the bottom of both cylinders. The tape will prevent the test tubes from sliding out of the cylinders. Make sure the tape is secure lest your test tubes drop out of the bottom of the cylinders.

(8) Rinse the inside of both test tubes and carefully clean the outside bottom of the test tubes.

(10) Using a plastic pipette, fill one of the test tubes with the stock solution to a depth of exactly 2.0 cm from the bottom of the test tube to the bottom of the meniscus of the solution. This will be the light path length, *b*, for the stock solution.

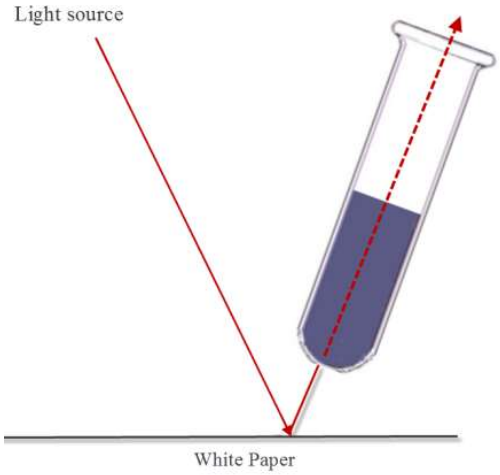


(11) Place the test tube with 2.0 cm of stock solution in one of the paper cylinders sliding it down to just touch the bottom of the paper cylinder so that it rests on the tape.

(12) Slide the second clean, empty test tube into the second barrel.

(13) Take a sheet of white paper and place it on a flat surface that has a white light shining on it. A lamp or a nearby window can be used for illumination. Alternately you could use the white screen of a tablet computer or even a cell phone as your source of white light. You just need a uniform source of white light that can project through the test tube solutions.

(14) Hold two test tubes so that you can view the lit paper or screen through both test tubes. The light should go through the length of the test tube with no light coming from the sides. That is why you needed to wrap the test tubes in paper.



(15) Using the graduated pipet, add solution A to the empty tube until it has the same degree of transparency as the 2.0 cm of the stock solution. The two solutions should have the same value (relative darkness) of color. This is not easy, since the test tube bottom is curved and produces a view that is like a bullseye. The center of the bullseye is the most accurate part to match. It's not easy but do the best you can. You need to adjust the depth of solution A so that looking through both tubes simultaneously they look identical, even though the solutions do not have the same concentration. The depth of the solution to compensates for the difference in concentration.

(16) Remove the test tube with solution A, from the paper cylinder. Carefully measure and record the height of solution A in the test tube, the light path length, *b*.

(17) Empty solution A and rinse the test tube with some solution B. Rinsing with water is not a good practice because any remaining rinse water in the tube would dilute any solution that you put in the test tube. It is good lab procedure to rinse glassware with the solution that will be placed in it for testing. If you can't dry a piece of glassware, which is often the case with tubes and flasks, rinse with the solution that will be put in the glassware rather than water.

(18) Match the transparency again this time with solution B.

(19) Repeat steps with solutions B through D.

Solution	light path length, *b,* for identical transparency
Stock Sol'n	2.0 cm
A	
B	
C	
D	

(20) Save your four solutions in the cups for the next set of labs.

Analysis

There is an Excel document at ChemAdvantage that will help you with these computations and graphs.

Make a graph plotting the inverse of the solution light path length, (1/*b*), on the y axis and the concentration on the x axis.

Develop an equation that relates the length of the light path for identical transparency to the concentration of the solution using one of the graphs of the data.
The Dubosq colorimeter[2] is a classic instrument used for over one hundred years to find the concentrations of colored solutions. It had a split lens viewer and used light paths to determine the concentrations of solutions. You can look over the instruction manual to see how this apparatus worked.

Just by putting two colored solutions in test tubes and looking at them, you could find the relative concentrations of the solutions. The equivalent instruments used today follow the same principles but use electronic light detectors to measure the amount of light rather than relying on human judgment.

One of the advantages of the electronic light detectors is that they can detect wavelengths of EMR that are not visible to humans. Most solutions that look transparent in visible light absorb "colors" in other parts of the spectrum.

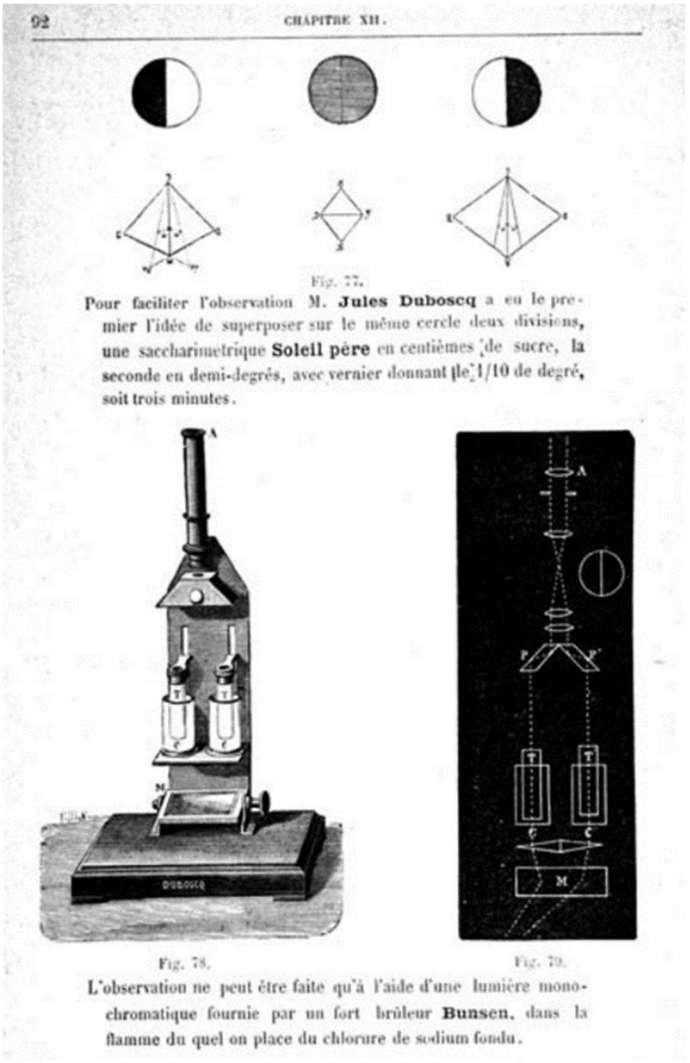


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Fig. 77.

Pour faciliter l'observation M. **Jules Duboscq** a eu le premier l'idée de superposer sur le même cercle deux divisions, une saccharimetrique **Soleil père** en centièmes de sucre, la seconde en demi-degrés, avec vernier donnant le 1/10 de degré, soit trois minutes.

Fig. 78. Fig. 79.

L'observation ne peut être faite qu'à l'aide d'une lumière monochromatique fournie par un fort brûleur **Bunsen**, dans la flamme du quel on place du chlorure de sodium fondu.

[2] Humboldt State University Robert A. Paselk Scientific Instrument Museum
http://www.humboldt.edu/scimus/HSC.36-53/Descriptions/Color_B%26L.htm

Colorimetry I Light Path

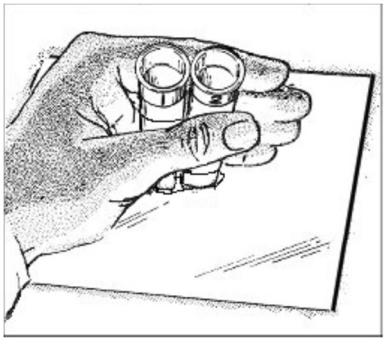
I made up a series of solutions in cups using a stock solution where I put three drops of blue food dye in 130 mL of water. I presumed that this solution would be 10.0 μM.

Then I used tared cups with 20 mL of stock solution and increasing amounts of water to make a series of solutions with lower concentrations.

$$Concentration_{DilutedSol'n} = \frac{20.\text{ mL}_{StockSol'n} \times 10.0\ \mu M_{StockSol'n}}{30.\text{ mL}_{DilutedSol'n}}$$

$$Concentration_{DilutedSol'n} = 6.7 \mu M$$

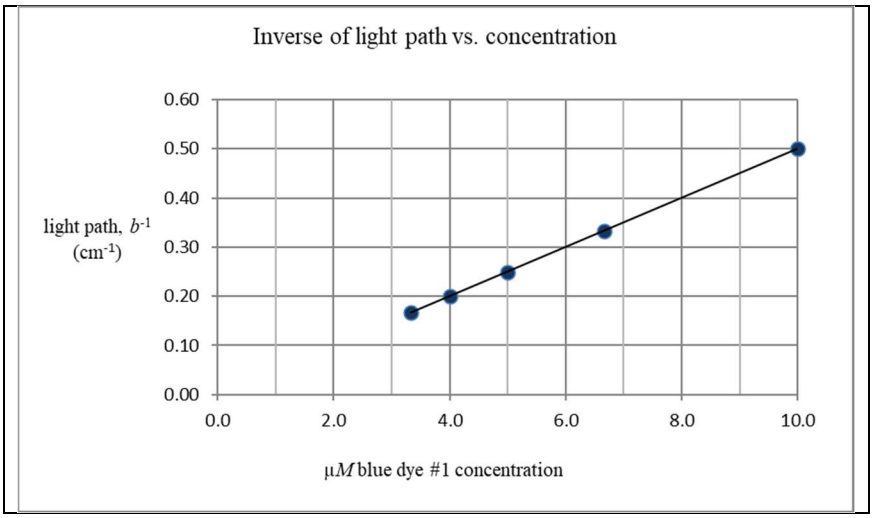
Solution Cup	Volume of stock blue dye solution (mL)	Volume of solution after dilution with water (mL)	Concentration Sol'n
Stock sol'n	20	20.	10. μM
A	20.	30.	6.7 μM
B	20.	40.	5.0 μM
C	20.	50.	4.0 μM
D	20.	60.	3.3 μM



Using the instructions in the lab guide, I made white cardboard taped cylinders to hold two test tubes so that I could view down the length of the tubes simultaneously against a uniformly lit white background.

I filled one tube to a depth of 2.0 cm with stock solution and added each diluted solution to the other tube so that when looking down the tubes the tubes looked identical. I needed to add more of the dilute solution than the stock solution to the second tube to get the same transparency. This was not easy because the round base of the test tubes produced a ring of color, but I was able to adjust the depth of the diluted solutions to get a good approximation. I then measured the depth of the diluted solution needed to get to the matching transparency. I then calculated the inverse of the path length.

Solution	Concentration Sol'n	light path length, b, for identical transparency	light path length, 1/b, for identical transparency
Stock Sol'n	10. μM	2.0 cm	0.50 cm^{-1}
A	6.7 μM	3.0 cm	0.33 cm^{-1}
B	5.0 μM	4.0 cm	0.25 cm^{-1}
C	4.0 μM	5.0 cm	0.20 cm^{-1}
D	3.3 μM	6.0 cm	0.17 cm^{-1}

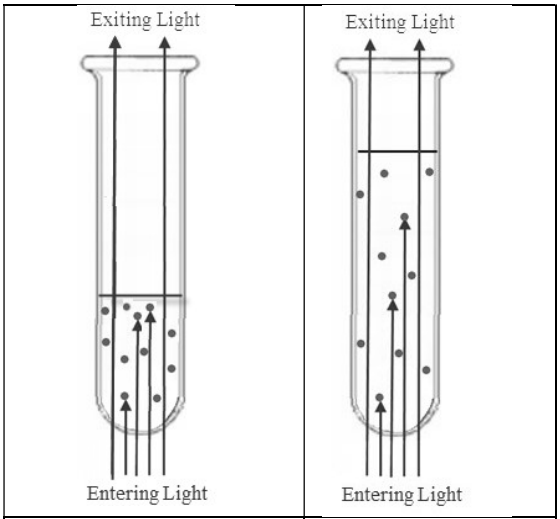


Equation: $b \times C_{solution} = constant$

Solution	*Concentration Sol'n*	*light path length, b, for identical transparency*	$b \times C_{solution} = constant$
Stock Sol'n	*10. μM*	*2.0 cm*	*20. μM cm*
A	*6.7 μM*	*3.0 cm*	*20. μM cm*
B	*5.0 μM*	*4.0 cm*	*20. μM cm*
C	*4.0 μM*	*5.0 cm*	*20. μM cm*
D	*3.3 μM*	*6.0 cm*	*20. μM cm*

Inverse path length graph is preferable because it produces a straight line whose slope is constant and also has a logical 0 point.

Comparative drawing



Stock Solution	*Solution D*

The stock solution D should have the same number of molecules with the same amount of light exiting and being absorbed by the molecules.