

Experiment

Colorimetry: Quantitative Analysis with Light

John R. Amend, PhD, Montana State University
and
Dale A. Hammond, PhD, Brigham Young University Hawaii

LEARNING OBJECTIVES

The purpose of this experiment is to . . .

1. learn to match the observed spectra of colored solutions with spectrophotometric charts of percent transmission and wavelength.
2. learn to predict the visible results of color mixing.
3. learn to make a set of solutions of known concentrations by dilution of a standard.
4. use colorimetry to determine the concentration of an unknown colored solution.
5. colorimetrically determine the concentration of chlorine in pool and tap water samples.

INTRODUCTION

Color is something that almost all of us take for granted. We cross streets according to directions from red and green lights. We are careful in the choice of our clothes, to make sure that the colors match. We paint our homes and automobiles to express both individuality and beauty. Artists attempt to capture the colors of a sunset or of autumn leaves.

Most of us believe that color is something that is emitted by "colored" substances. Color, however, is caused by exactly the opposite effect. White light consists of all colors of the visible spectrum from violet to red. Colored substances selectively absorb some of the colors comprising the white light, and ultimately turn it into heat. The remaining colors of light are reflected or transmitted by the sample, and we see them as the "color" of the material.

Chemists have learned to use this selective absorption of light to quickly determine the concentration of solutions of colored substances. If light of a color (or wavelength) absorbed by a material is passed through a sample, the amount absorbed will be proportional to the number of colored light-absorbing molecules in the light beam. One can construct a calibration graph by measuring the transmission or absorption of light in a series of samples, starting with distilled water as a zero concentration sample, and proceeding to more concentrated solutions. This calibration graph then can be used to determine the concentration of unknown samples. Analytical chemists, particularly in the agricultural and medical fields, routinely use this technique

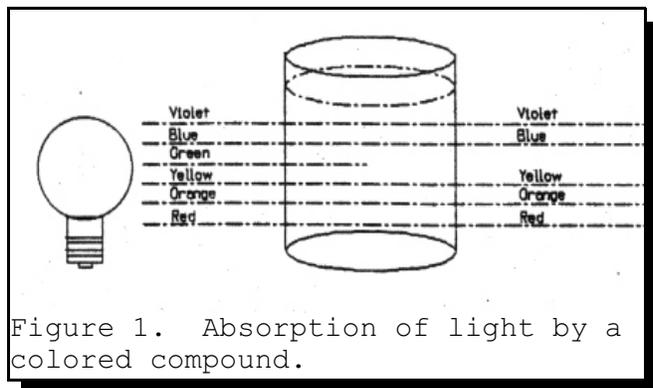
for determination of concentration of various materials in inorganic and biological samples. For example, the concentration of iron in blood samples is almost always determined colorimetrically.

This experiment comprises three sections. Part One involves some demonstrations of color and color mixing that your laboratory instructor will work through with you on the overhead projector. Part Two involves development of a calibration graph and determination of the concentration of an unknown sample of food coloring. Part Three will apply this technique to determination of the concentration of chlorine in samples of pool and tap water.

PART 1: THE FORMATION OF COLOR

Part One of this experiment involves a series of demonstrations using an overhead projector that your laboratory instructor will perform with you.

Problem 1: The formation of color



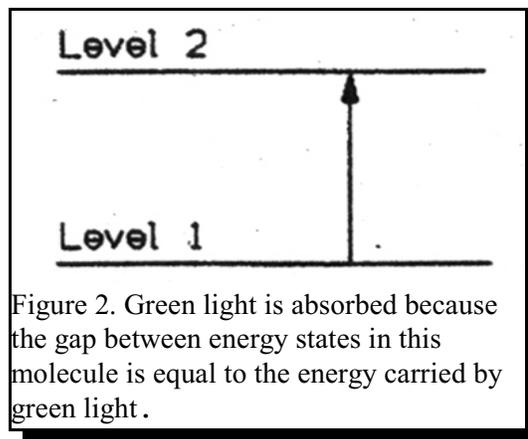
A very dilute solution of potassium permanganate in a petri dish will be placed on the overhead projector, and you will observe the spectrum of this sample using a diffraction grating. Potassium permanganate forms a purple solution. Note, however, that the projection spectrum of the solution shows transmission of violet, blue, some yellow, orange, and red. Green and some yellow light is selectively absorbed.

When light of the proper energy strikes an electron, the electron can be raised to a higher level of energy, and the light will be absorbed. If a spectrum of white light strikes a colored sample, only those colors of light that exactly match possible electron transitions in the sample will be absorbed. In the case of the potassium permanganate solution, green and yellow light are selectively absorbed. The electron jumps possible in potassium permanganate are the same energy as the energy carried by green and yellow light.

Problem II: Spectral Transmission Charts

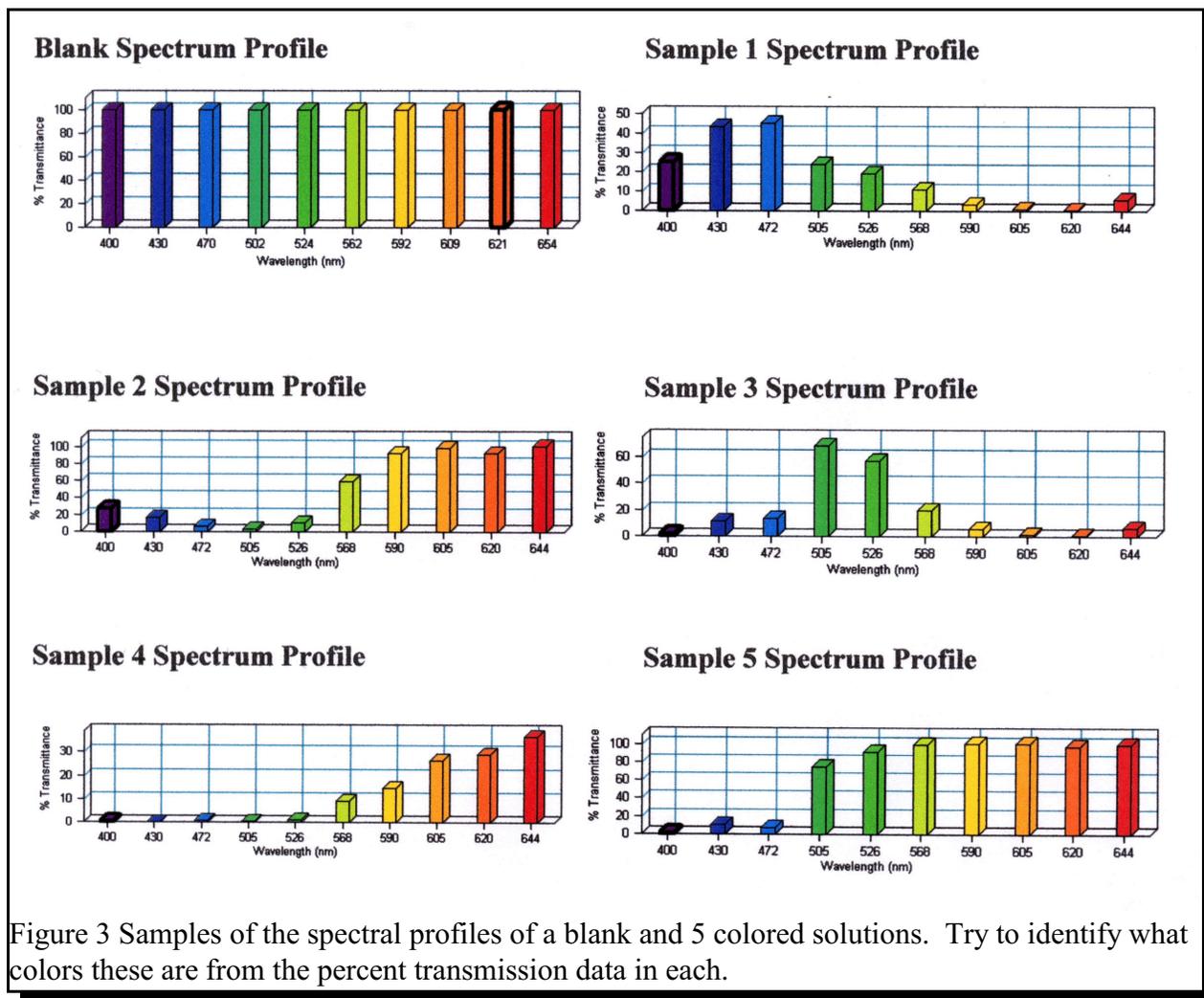
The charts on this page correlate percentage transmission with color and wavelength. These charts are for the following substances: a comparison blank, red, yellow, green, and blue food colorings, and potassium permanganate. These charts, however, are not labeled or arranged in any particular order.

Your instructor will display samples of each substance on the overhead projector, where you will view the absorption



spectrum of each sample, and identify each chart with the proper substance. Take careful notes on this as this identification will be very important for the next section of this experiment. **Be sure to identify the color of these by sample number in your report.**

PART 2: COLOR MIXING ON THE MICROLAB INTERFACE.



Connecting the Colorimeter:

1. Obtain the MicroLab interface with its built in colorimeter, and a corresponding numbered power supply box. Plug in the power transformer to the wall socket, then plug the other end into right back of the interface.
2. On the computer, click on the **MicroLab icon** In the opening screen, click on the **Color Mixer Experiment icon**, and if necessary, click in the middle box in the upper right corner to expand the window full screen.

3. Insert a **Blank** (a vial containing only water) into the colorimeter chamber, make sure the lid is on tightly and click on the word "**Blank.**" The lights in the **Colorimeter** section of the front panel will blink for about 10 seconds, then a bar chart will appear in the top right window showing all wavelengths at 100% transmission. The program will then shift to the second upper left tab, **Read Samples**.
4. Change the blank vial for one of the colored solution vials, and click on Add. (Always make sure the lid is on tightly.), Type in the color name, then press **OK** to measure the transmission of that solution. The bar graph in the top window will then change to reflect the transmission of the 10 wavelengths of that solution.
5. Repeat step four for each of the remaining four solutions to obtain the 10 wavelength spectrum of each solution.
6. Click on **Tab 3** to go to the **Mix Colors** section, then chose two colors to mix by clicking on the arrow box at the side of windows **Color 1** and **Color 2** and then clicking on the desired colors. The first color selected will appear in the top window on the right, and the second color will appear in the middle window on the right.
7. Examine each of these bar graphs carefully, looking at both transmittance and absorbance, to ascertain how they will add together when they are mixed, then in your lab notes draw a rough graph of the spectrum profile to show what you think the result will be.
8. Now click on the **Show Mixing Controls** button, then click on the **Mix** button to see what the actual result will be, then compare this **mixed bar graph** with the actual colors by holding the two selected colored cuvettes up to the light, with one in front of the other. Compare the color you seeing versus the colors absorbed and transmitted.
9. Repeat this process for the remaining colors, and construct a table of your data listing the two colors mixed, a sketch of your predicted graph, a sketch of your actual graph, and the color you saw for the mixed colors (from the two vials side by side).

Comparing Colors

With the **Color Mixer** program, it is possible to compare two colors to see if they are the same. This type of comparison is often done in forensics (detective) work, for example, to see if fabric fibers have the same dye composition.

When you mixed the blue and yellow food dyes together, you obtained a green color. In the original colored solutions you were given, you also had a green solution. Let's compare these two solutions to see if they are the same.

1. Select the blue and yellow colors in windows 1 and 2 as was done above.
2. Mix the blue and yellow colors, as above, and the mixed image will go into window 3.

3. Click in window four and select the original green color scanned in. When you have selected this color, the program will overlay the original green to the right of the mixed green and you will be able to compare their transmittance or absorbance wavelength by wavelength.
4. If the absorbencies do not match up, move the slider up and down to change the mixing percentage ratio of the blue and green colors in the mixed color graph.
5. If the two graphs can be made to match, then the original green color is a mixture of the blue and yellow colors, and thus from the same compounds. If they cannot be made to match, then they are different compounds.
6. **Print this screen** out to hand in with your report and **indicate on the report** if the green color is the same as the mixture of blue and yellow.

Note 1: To print the entire screen, use **ALT-Print Screen** to capture the screen, then open **WordPad** and do a **Ctrl-V** to copy the screen into **WordPad**, then do **Ctrl-P** to print the **WordPad** document. **You can combine two color mixing printouts per page, one after the other, to conserve paper.** Close out the **Color Mixer** program when finished.

PART II: COLORIMETRY

It is relatively easy to compare the relative concentrations of colored solutions. One could, for example, readily identify dilute and concentrated samples of Kool Aid by looking at the depth of color. *Colorimetry*, the measurement of the concentration of solutions by measuring their absorption of colored light, is quick, clean and quite accurate.

Colorimetry is more complicated than it looks at first glance, however. White light is composed of all of the colors of the visible spectrum - red, orange, yellow, green, blue, and violet. Colored solutions are colored because they absorb one or more of these colors, and let the others go through. A purple solution, as we saw earlier, absorbs green light (Figure 1). Our eye sees red, orange, yellow, blue, and violet light passing through the solution. Change the blank vial for one of the colored solution vials and our brain interprets this as purple. Red solutions are red because they absorb violet, blue, and green light, and transmit yellow, orange, and red light. Blue solutions transmit blue and green light, but absorb yellow, orange, and red light.

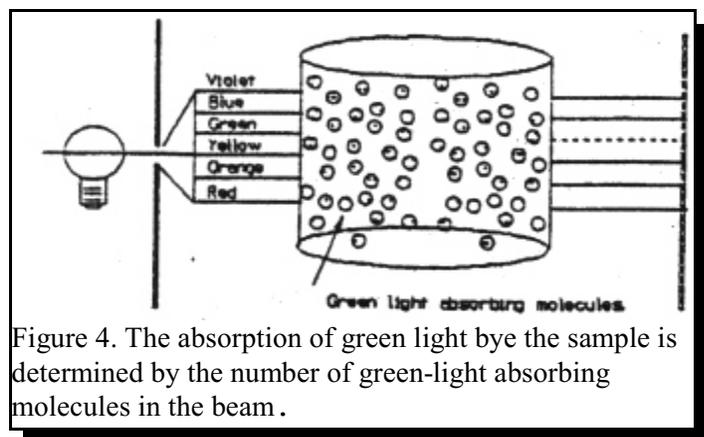


Figure 4. The absorption of green light by the sample is determined by the number of green-light absorbing molecules in the beam.

This phenomenon can be used to measure the concentration of a colored solution. Suppose that one had a red solution. The solution is red because its molecules absorb green, blue, and violet light. If red light were transmitted through the solution, there would be little absorption. However, if green light is transmitted through the solution, each time a photon of green light struck a green-light absorbing molecule, it would be absorbed. Thus the number of photons of green light that will make it

through the solution is inversely proportional to the number of green-light absorbing molecules in the light beam

There are two ways we can get more green light-absorbing molecules in the light beam. One way is to make the sample container longer. The other way is for the path length of the light to be the same, but for the solution to be more concentrated.

Unfortunately, the absorption of colored light is not linearly related to the number of light absorbing molecules in the beam. Consider the situation in which a 1 cm cell contains enough green-light absorbing molecules to absorb half of the light that strikes the cell. Figure 5 shows four examples of this.

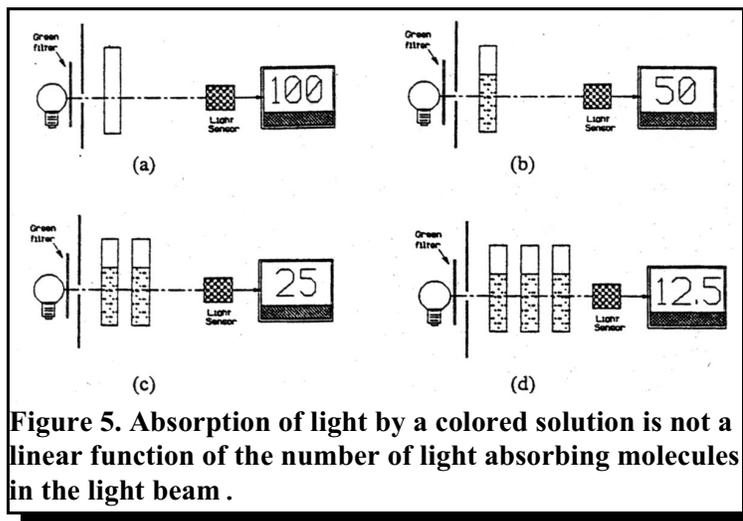


Figure 5. Absorption of light by a colored solution is not a linear function of the number of light absorbing molecules in the light beam .

In example (a), a blank cell (filled with distilled water) is present, and 100% of the incident light passes through the cell. In example (b), this cell has been replaced with a similar cell with enough green-light absorbing molecules that $\frac{1}{2}$ of the light is absorbed. A detector placed on the other side of this cell will read a transmission of 50%, as compared to the distilled water blank.

In example (c) a second cell containing the same colored solution is placed in the beam. This cell absorbs half of the green light that strikes it. A detector placed on the other side of this cell will read $\frac{1}{2}$ of the incident light striking this cell, or 25% of the initial light.

Example (d) shows three cells of the same colored solution. Again, $\frac{1}{2}$ of the light striking cell number three will be absorbed, and the detector will read 12.5% of the initial (blank) light reading.

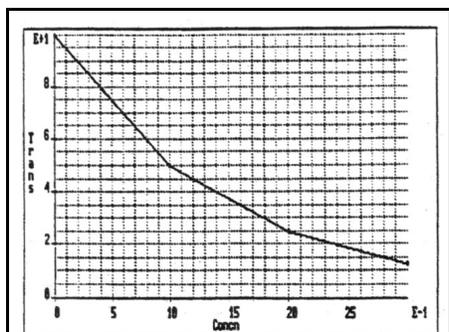


Figure 6. A graph of the number of light-absorbing molecules in a colored light beam makes a smooth curve when compared to the transmission of light through the sample .

A graph of the results from the experiment illustrated in figure 5 is presented in Figure 6. In this graph, concentration is plotted on the horizontal or "x" axis, and percent transmission on the vertical or "y" axis.

Note that a smooth curve results, with 100% transmission at zero concentration, and 12.5% transmission with three of the solution cells in the light path.

One could obtain exactly the same effect by increasing the concentration of the colored molecules in a single 1 cm cell. The concentrations of this example might be zero (the blank), 1 unit, 2 units, and 3 units.

Curves, however, are difficult to use for predictions. If one plots the *logarithm* of the transmitted light against concentration, this time a straight line results on the graph. *Semi-logarithmic* graph paper makes this an easy task. Figure Seven presents this plot. Percent transmission data is plotted on the vertical, logarithmic axis, while concentration is plotted on the horizontal, linear axis. From this calibration chart, one can relate a concentration value with any measured percent transmission from zero to 100 percent.

USE AND CALIBRATION OF THE MICROLAB COLORIMETER:

With the MicroLab interface set up as discussed in Part 2 above, do the following:

1. Open the *MicroLAB* program as before, and click on the **Colorimeter Experiment icon**. Click in the middle box in the upper right corner to expand the window full screen, if necessary.
2. Insert a **Blank** (a vial containing only water), make sure the lid is on tightly, and click on the word **“Blank.”** The lights in the **Colorimeter** section of the front panel will blink for about 10 seconds, then a bar chart will appear in the top right window showing all wavelengths at 100% transmission.
4. Click on **Add Data**, change the blank vial for the 100% standard vial, **always make sure the lid is on tightly**, type in the color name, e.g., Blue 100, then press **Tab** key to shift the cursor to the next window, and enter the concentration of that standard, e.g., 10.0, then press **OK** to measure the transmission of that solution. The bar graph in the top window will then change to reflect the transmission of the 10 wavelengths of that solution. **All concentrations may be entered as the volume used to make that solution or the percentage of the concentrated solution.**
5. Repeat step five for each of the remaining standards to obtain the 10 wavelength spectrum of each standard.
6. Click on **Absorbance** at the top of the screen, select the appropriate wavelength for doing the analysis by choosing that wavelength where the absorbance is greatest, and write down the equation of the Beer's law regression line in your lab notes.
7. Now click on the **Unknowns** tab and measure the absorbance of each of your unknown solutions. As you do this, the **Colorimetry** program will calculate the concentration of each unknown from the

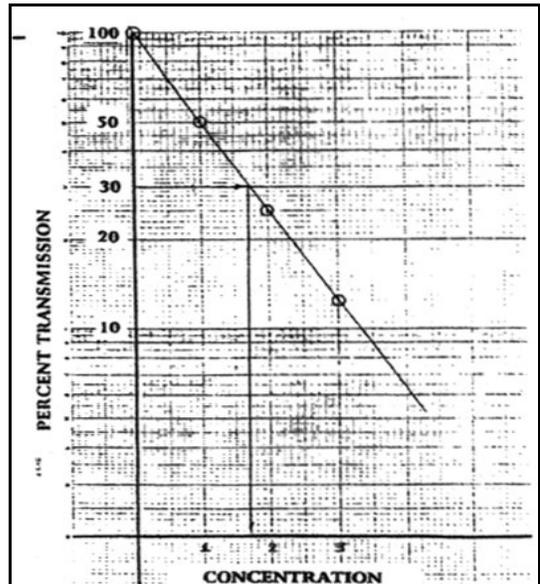


Figure 7. A semilogarithmic calibration graph of percent transmittance vs concentration permits one to determine the concentration of unknown colored solutions

regression equation at the bottom of the regression graph, and will also enter the data into the data table.

- When all of your unknowns have been measured, print out the screen by using the **ALT-Print Screen** process discussed above in **Part 2**. **You can print two of these pages per 1 printout to conserve paper. Be sure to include these copies in your report.**

PREPARATION OF STANDARD SOLUTIONS FOR CALIBRATION:

There are many different units of concentration. Most of these report the number of parts of sample per unit volume of the solution. For our first experiment in colorimetry, we will use “Units of Blue”, abbreviated “UB”, for the number of blue food color units per 10 units of solution”. We can make up our standards with a **clean** 50 ml buret, a 10 ml volumetric flask and a small plastic pipette with which we can add drops of sample or water. Table 2 shows how to mix up these samples. These standards should be made with the utmost care to insure their accuracy, and the solutions should be mixed thoroughly in the volumetric flask.

A FOOD COLOR UNKNOWN:

Use the MicroLab to measure the absorbance of each of your standards, and unknowns and print out the data as above.

PART III, APPLICATION: DETERMINATION OF THE CHLORINE CONCENTRATION IN POOL AND TAP WATERS

BACKGROUND

Chlorine and chlorine compounds are commonly used in disinfecting drinking water, pool water and waste water in different forms. Controlling the amount of chlorine in the water sources is important due to the harmful effects that excess chlorine can cause to both humans and to water ecology. For the purposes of this experiment, we are only going to concern ourselves with the low range of chlorine in pool water and tap water samples.

ANALYSIS FOR CHLORINE:

The procedures used to find the chlorine concentration of pool and tap water are almost identical to the procedures used to find the unknown food coloring solution. The only difference is you will make 10 standard solutions, and you will treat your samples with an ITS test strip for free chlorine to develop a color in the solutions proportional to the chlorine concentration to make it visible to the colorimeter.

- The standard chlorine solution will be considered to be parts per million (ppm) of chlorine, so you should make four dilutions to this in the same manner as you did for the blue food dye, such that the solutions will be 20, 40, 60 and 80 percent as concentrated as the standard. You

Table 2
Preparation of Calibration Solutions

mL food color	mL water	Concentration
0	10	0 parts/10 mL
2	8	2
4	6	4
6	4	6
8	2	8
10	0	10

should label each of these in ppm of chlorine. Use the pipette and a 10 mL volumetric flask to make these solutions. **MAKE THESE STANDARDS VERY CAREFULLY OR YOU WILL NOT GET GOOD RESULTS.** Be sure to record the volume of the standard solution added, the volume of water added, the resulting concentration, the %T and the absorbance in your laboratory notebook in a table similar to the one above. **Be sure to use de-ionized water to make your solutions.**

2. After diluting the standard solution, you should have six solutions to measure: the undiluted chlorine standard, four dilutions and the pure de-ionized water. Keep each solution in a well-labeled container.
3. All eight of the solutions (the six standards, the tap and pool water) should be made up and be transferred to colorimeter vials before proceeding with the next step. Run the tap water for about five minutes before measuring out 10 mL.
4. Get a new **Colorimeter Experiment** and run the **Blank** with de-ionized water. Obtain seven ITS Free Chlorine test strips and, one at a time, place the vial in the colorimeter, place an ITS test strip in the vial and swish it back and forth and up and down to ensure thoroughly mixing for 30 seconds, then place the cap on the colorimeter and take the measurement. The solutions should turn various shades of red, depending on the amount of chlorine in each solution.
6. After measuring the standard solutions, click on the **Absorbance** button, do a linear best fit or a quadratic fit, whichever gives the best correlation, then measure the samples of pool and tap water.

DATA ANALYSIS

As indicated above, too strong a chlorine concentration causes the red coloration to be bleached, giving rise to a non-linear relationship, even when plotted logarithmically. The more concentrated the chlorine, the more bleaching that occurs. Consequently, for this portion of the experiment, we will use a different method to determine the concentration of the chlorine than we did with the blue food coloring. We usually try to linearize all data graphs in order to be able to determine the physical relationships involved in the experiments. In this experiment, we know the physical relationship already, i.e., $\text{Conc.} = 2 - \log(\%T)$. Our purpose here is to obtain the best value possible for the chlorine concentrations of the unknowns. Consequently, we will use the curve fitting capability of *MicroLab* to do a second order polynomial fit to the data, then use the predict function to determine the concentration.

1. Print your Colorimeter screen out as discussed above, using the **CTRL-Print Screen** function.
2. Include the absorbance and concentration values from the data section for the concentration of the pool and tap water information in your lab notebook and on your data sheet for your report, along with the linear curve fit or the quadratic equation and the correlation for each Beer's plot.

REPORT:

Write a brief report in the format discussed by your instructor. Be sure you provide all of the printouts requested, and fill in the tables on the report sheets, which should be integrated into your report, along with any discussion needed.

