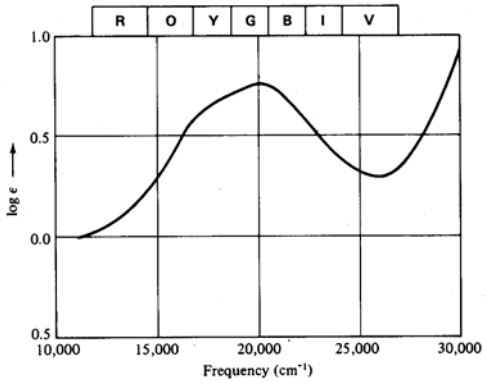
> He that will not apply new remedies must accept new evils: for time is the great innovator
> --Francis Bacon

Solution Concentration and Absorbance

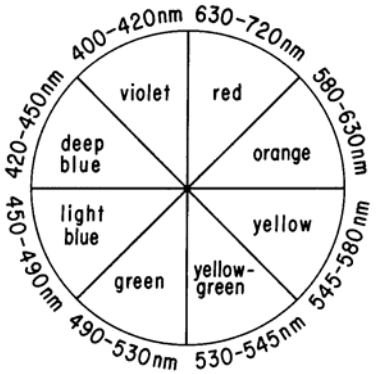
In your earlier studies you have already seen that the concentration of a solute in a solvent can be determined in a variety of ways: volumetric and gravimetric. Titration and recovery of precipitates are two very basic and useful techniques. It would also be possible to employ colligative properties to the same end. This experiment concerns another technique that is applicable to solutions which *absorb* visible light.

[**transmittance** is the ratio of the light intensity exiting a sample compared to the light intensity entering a sample; absorbance is the negative log of *transmittance*, i.e.,
A = -log T OR A = 2-log %T]

Most solutions absorb electromagnetic radiation of some wavelength. Those which appear colored to the human eye absorb in the *visible* range of the spectrum. The theories behind the production of color in solution can be pretty complex but you might suspect that electron transitions of some sort are involved. In fact, these *quantized* transitions often account for relatively sharp absorption spectra (within narrow wavelength ranges). Consider the absorption spectrum of $Ti(H_2O)_6^{3+}$:



Consulting a wavelength table you would find that the maximum absorption is in the yellow-green region. But the solution itself appears *violet*. This is because when yellow-green light is removed from the mixture of visible light we call "white", the remaining colors mix to give violet. In general, the apparent color of a solution is the *complement* of the color of light which is absorbed (see the following diagram on which complementary colors are opposite one another)



Before you get the idea that the situation is fairly simple you should also know that many solutions absorb at more than one wavelength although they appear to be just one color. This should not be surprising since electrons exist on many energy levels in atoms and molecules and can be expected to make a variety of transitions involving different energies and so absorbing different wavelengths (remember, $\Delta E = hc/\lambda$).

This would just be very interesting if not for the fact that absorbance is proportional to concentration! Strictly speaking that proportionality is expressed as *Beer's Law*:

$$A = \varepsilon bc$$
$$y = mx + b$$

where **A** is the absorbance (unitless), **ε** is a proportionality constant known as the *molar absorptivity coefficient*, **b** is the path length of solution through which the light travels (in cm), and **c** is the concentration of the solution (in Molarity). This is a linear relationship and is generally true for most solutions. Like most "laws" there are exceptions and solutions which behave most ideally are those which have moderate concentrations and are examined *at the wavelength of maximum absorbance*. Because some ordinary solutions can deviate from Beer's Law, it is common practice to develop a *calibration graph* for a particular system before interpreting the concentration of a solution based on its absorbance. The calibration graph is (hopefully) a straight line which relates absorbance to concentration and is obtained by measuring the absorbance of a number of samples with known concentration and plotting the results. The concentration of an unknown solution can then be read from the graph if its absorbance is measured.

Although relative absorbances can be estimated with the unaided eye, most measurements are done with instruments. There are about three different kinds. A *spectrophotometer* can be adjusted to produce one wavelength (the one at which the sample most strongly absorbs). A *recording spectrometer* scans the visible range and measures the absorbance of the sample throughout.

This results in a "fingerprint" much like the spectrum of $Ti(H_2O)_6^{3+}$ given earlier. Finally, a *colorimeter* uses some kind of filtering device to produce light that is roughly an approximation of the wavelength of strongest absorbance. This is the least expensive device. In each device the sample is placed in a *cuvette* which is optically clear and has a fixed path length (eliminating that variable). Light is passed through the sample and the intensities incoming and outgoing are compared. This information is displayed either as % Transmittance or Absorbance.

The device you will be using connects to the CBL and is a hybrid between the first and the third type of devices. It can produce light in three narrow ranges of the visible spectrum. For this experiment the solution used is blue and the closest complementary color available is RED. Distilled water is used to zero the colorimeter (100% transmittance) and then the transmittance is read directly off the CBL screen as you check the samples.

Preparing to experiment

You will be provided with the following materials:

1. a colorimeter probe
2. a standard solution of "0.50 M" Blu
3. a sample cuvette
4. kimwipes
5. 1 mL calibrated beral pipets
6. a Blu solution of unknown concentration

Design a procedure to prepare at least 3 known concentrations of Blu solution in addition to the standard solution. (hint: make dilutions of the standard solution with distilled water, 3 mL total volume for each mixture)

Design an experiment to construct a calibration graph for Blu using the RED light and determine the concentration of the unknown Blu solution.

BE SURE TO BRING YOUR TI-83/P CALCULATOR TO CLASS FOR THIS EXPERIMENT. YOU WILL ALSO NEED A COPY OF THE HCHEM.83G FILES IN YOUR CALCULATOR MEMORY.

Pre-lab take-home quiz

These questions should be answered on a separate sheet of paper to be turned in on the day you do this experiment.

1. A Ni^{2+} solution absorbs strongly in the red region of the visible spectrum. What is probably the color of the solution?

2. A nickel(II) solution with a concentration of 0.25 M has an absorbance of 0.32. Assuming that this solution obeys Beer's Law exactly, what must be the concentration of another nickel(II) solution with an absorbance of 0.13? (assume *b* is constant)

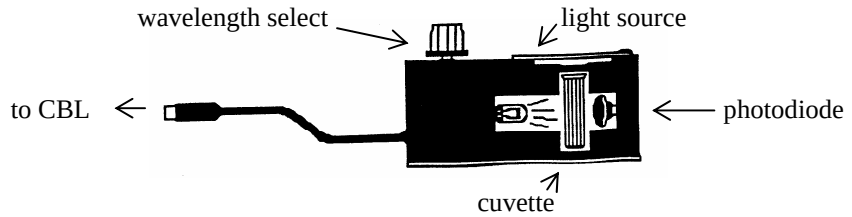
Technique

1. the solution

The solution you are using in this experiment is distilled water with blue food coloring added. Although a more traditional experiment might involve a copper(II) solution (also blue in color), there is no reason to use copper compounds just to end up throwing them down the drain. The blue colored water was prepared so that its absorbance matched that of a 0.50 M copper(II) solution. In determining the "concentration" of diluted samples, you can use this value as the concentration of the original solution since the dilution calculations will yield proportional values for the "Molarity" of the samples you prepare.

2. using the colorimeter probe

The colorimeter probe is a simple device which is diagrammed below:



The wavelength select knob has a setting for 0% transmittance which is used during the calibration routine run by the program in your calculator. The light selection is also made with this knob which is turned to the wavelength (color) that will be most strongly absorbed by the solution.

The colors available are red (635 nm), green (565 nm), and blue (470 nm). Each color actually covers a small range but the most intense wavelength in the band is given in parentheses. There is also a cover that should be in place over the cell to prevent stray room light from affecting the reading.

In order to obtain the most reliable results, you need to know what color of light is most strongly absorbed by the sample. Based on the color wheel shown at the beginning of the experiment, RED is the best choice for this experiment.

Because the colorimeter draws a significant amount of power from the CBL to run the lights, best results are obtained if the AC power pack is used with the CBL whenever the colorimeter is in use.

When you set up the calculator for this experiment you will be using the third probe option, % TRANS (or % transmittance). Once you have made this selection you will automatically begin the calibration sequence which involves setting the "zero" reading for your colorimeter using distilled water. Follow the instructions on the calculator screen. Once this is done you will be returned to the main menu to select the method for collecting data.

You can collect data in CONTINUOUS mode for this experiment. You will then record the % transmittance by hand and later convert it to absorbance before graphing. Don't forget that the "blank" (distilled water) is one of your samples (with a Molarity of 0.0).

There is another option, however. If you use the VS. USER X option for collecting data, you can store your readings in the calculator memory. Later you can use the PROCESS DATA option from the main menu to convert your readings to absorbance automatically (and then graph, if you like). There are two useful options available under VS. USER X. You can specify that the X values will just be sample numbers (1, 2, 3, et.). In your data table you would need to keep a list of which sample is what. OR you could specify the X values as Molarity. This means you would have to calculate in advance what Molarities you plan to prepare with your dilutions. This last option would be the most efficient since all of the correct data will be in the calculator when you are finished, ready to graph. The only drawback is that you can't do your unknown this way since you don't know its Molarity!! There are at least two ways to deal with this problem: you can exit from the routine after your last sample, return to the main menu and select CONTINUOUS to read your unknown OR you can read the % transmittance of your unknown while the CBL is sampling, write down the value, then replace the unknown with the next sample before using the [TRIGGER] button to record the value. And don't forget that the "blank" (distilled water) is one of your samples (with a Molarity of 0.0).

3. cuvettes

The sample holders or cuvettes are made of plastic and can be easily scratched so you should handle them with care. For the best results you should use the same cuvette for each reading and you should always insert the cuvette into the colorimeter the same way (i.e., the same side facing the photodiode). Following these protocols eliminates the need for sets of "matched" cuvettes which are optically identical. Your cuvettes have an ink mark on the top edge to use as a reference for consistent placement in the colorimeter.

It is necessary to rinse and dry the cuvette between samples. Wrapping a lintless kimwipe around your stirring rod can help to dry the inside. Be sure the rod is completely covered so you do not scratch the cuvette. To ensure that no liquid gets in the colorimeter and that fingerprints are removed from the cuvette, use a kimwipe on the clear sides of the cuvette each time before placing it in the probe (one towel can be used more than once).

4. Pipets

You have used graduated cylinders in the past to measure volumes of solution. One step beyond the graduated cylinder in terms of precision is the *pipet*. A pipet looks like a long eye dropper without a bulb. There are two basic types of pipets. One is *volumetric*. This has ONE mark on it and is good for repeatedly measuring out a single volume. A *measuring* pipet, on the other hand, is very much like a graduated cylinder. It has many marks and comes in many sizes. In this experiment you will use a graduated beral pipet. The stem of the pipet is graduated for 1 mL in 0.25 mL units. While not as accurate as a glass measuring pipet, it is sufficient for the purposes of this experiment and allows us to use small amounts of solutions in a relatively short amount of time.

For the most accurate use of the beral pipets it is probably easier to fill the bulb and expel whatever volume of solution you don’t want back into the bottle. As with any measuring in a graduated device, the liquid level should be ascertained at eye-level.

To simplify your measuring we will supply you with labeled pipets, one for water and the other for Blu. Be sure to observe these labels.

Analysis

1. Use your volume data to determine the concentrations of the dilutions you prepared from the standard Blu solution. [recall, $V_1M_1=V_2M_2$ where V_1 is the initial volume of the solution with concentration M_1 and V_2 is the <u>total</u> volume of the new solution with concentration M_2]

2. Convert your % transmittance data to absorbance for each sample. Don't forget the distilled water!

3. Use your absorbance data and the concentrations from #1 to plot a calibration graph for the Blu solution. Don't forget to include the "blank" (Molarity = 0.0, absorbance = 0.0). Draw the best-fit line.

4. Determine the concentration of your unknown Blu solution from the calibration graph.

5. Does your best-fit calibration line actually pass through 0,0? Should it? Why or why not?