Colorimeter

(Order Code COL-DIN)

The Vernier Software Colorimeter is a computer-interfaced probe designed to determine the concentration of a solution by analyzing its color intensity. The color of a solution may be inherent or derived by adding another reagent to it. Monochromatic light from a LED light source passes through a cuvette containing a solution sample, as shown in Figure 1. Some of the incoming light is absorbed by the solution. As a result, light of a lower intensity strikes a photodiode.

NOTE: This product is to be used for educational purposes only. It is not appropriate for industrial, medical, research, or commercial applications.

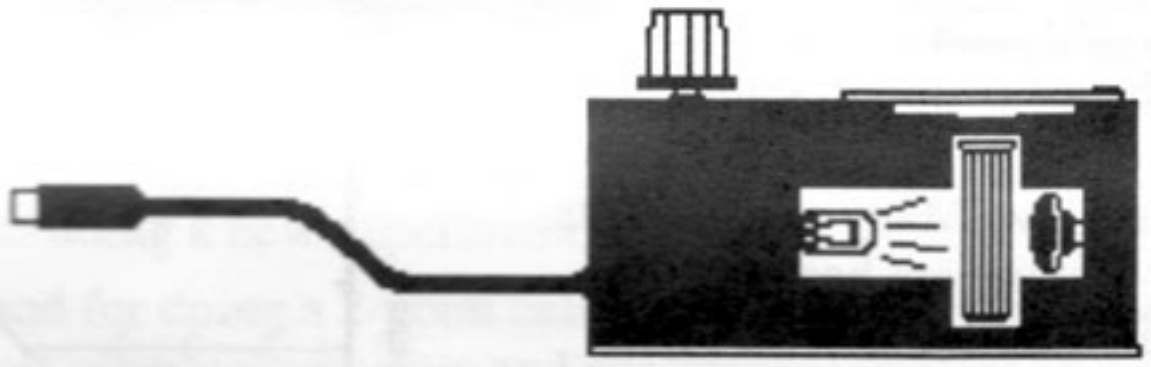


Figure 1

Transmittance and Absorbance

The amount of light that penetrates a solution is known as *transmittance*. Transmittance can be expressed as the ratio of the intensity of the transmitted light, I_t, and the initial intensity of the light beam, I_o, as expressed by the formula:

$$T = I_t / I_o$$

The Colorimeter produces an output voltage which varies in a linear way with transmittance, allowing a computer to monitor transmittance data for a solution. The transmittance of the sample varies logarithmically (base ten) with the product of three factors: ε, the molar absorptivity of the solution, b, the cell or cuvette width, and C, the molar concentration.

$$\log(1/T) = \varepsilon bC$$

In addition, many experiments designed to use a colorimeter require a related measurement, *absorbance*. At first glance, the relationship between transmittance and absorbance would appear to be a simple inverse relationship. That is, as the amount of light transmitted by a solution increases, the amount of light absorbed might be expected to decrease proportionally. But the true relationship between these two variables is inverse *and* logarithmic (base 10). It can be expressed as:

$$A = \log(1/T)$$

Combining the two previous equations, the following expression is obtained:

$$A = \varepsilon bC$$

In effect, this formula implies that the light absorbed by a solution depends on the absorbing ability of the solute, the distance traveled by the light through the solution, and the concentration of the solution. For a given solution contained in a cuvette with a constant cell width, one can assume ε and b to be constant. This leads to the equation:

$$A = k \cdot C \text{ (Beer's law)}$$

where k is a proportionality constant. This equation shows absorbance to be related directly to concentration and represents a mathematical statement of Beer's law. Beer's law is discussed in more detail on the next page.

In this guide and in our computer programs, transmittance is expressed as percent transmittance or %T. Since T = %T/100, the formula can be rewritten as:

$$A = \log(100/\%T) \text{ or } A = 2 - \log\%T$$

Two of the Vernier Software programs, Data Logger and Spectrophotometer Program, are designed to calculate the absorbance from the percent transmittance data. Voltage Plotter and MPLI Program, however, read just percent transmittance data from the Colorimeter. The absorbance must be calculated independently of the program, using either of the formulas shown above.

Beer's Law

In general, absorbance is important because of its direct relationship with concentration according to Beer's law. Many experiments in chemistry and biology are based on this concept. To obtain a Beer's law curve, several standards (solutions of known concentration) are prepared and their absorbance values are determined using a colorimeter. A graph of absorbance versus concentration is then plotted. A solution of *unknown* concentration is placed in the colorimeter and its absorbance measured. When the absorbance of this solution is interpolated on the Beer's law curve, as shown in Figure 2, its concentration is determined on the horizontal axis. Alternatively, its concentration may be found using the slope of the Beer's law curve.

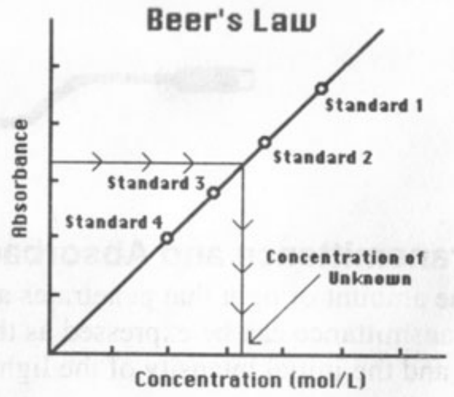


Figure 2

Absorbance and Transmittance Ranges for the Colorimeter

For best results, our laboratory testing of the colorimeter indicates that absorbance or transmittance values should fall within these ranges:

percent transmittance:	28% – 90%
absorbance:	0.050 – 0.550

We have found that Beer's law curves started to lose their linearity at absorbance values above 0.550 (percent transmittance values less than 28%). If you have a solution that transmits such a low level of light, consider diluting the solution so that it falls within this range. Try to design experiments so that absorbance or transmittance values of solutions are in this range.

Wavelength Ranges

You can select three LED light colors with the Vernier Software Colorimeter: Blue (470 nm or 4700 Å), green (565 nm or 5650 Å) and red (635 nm or 6350 Å). You can select one of these three nearly monochromatic colors using the wavelength selection knob on the top left of the colorimeter (see Figure 3). There are several ways you can decide which of the three wavelengths to use.

- Look at the color of the solution. Remember that the color of a solution is the color of light which passes through it. You probably want to use a different color of light that will be absorbed, rather than transmitted. For example, with a blue $CuSO_4$ solution, use the red LED (635 nm).
- Another quick method is to place a cuvette containing the solution in question in the Vernier Colorimeter and check to see which of the three wavelengths yields the highest absorbance (or lowest transmittance).
- Directions for most colorimetry experiments express a recommended wavelength. Use the closest of the three wavelengths on our colorimeter. Even if the LED wavelength is somewhat different, a Beer's law curve can usually be obtained at almost any wavelength in the vicinity of the recommended wavelength.

Calibration of the Colorimeter

We recommend you do a new calibration any time you perform a new colorimetry experiment or change the wavelength within an experiment. Though it is possible to save a calibration for future use, you will certainly see improved results if you recalibrate prior to doing a new experiment.

The general method for doing a 2-point calibration with the Colorimeter is similar to that used with most other colorimeters and spectrophotometers. A zero percent calibration is done with no light passing through a cuvette. The wavelength knob on the colorimeter is turned to "0% T" (see Figure 3). In this position the computer can read data from the Colorimeter, but the light source is turned off. Since the light is off, it makes no difference if a cuvette is in the cuvette slot. A 100% calibration is done with the wavelength knob turned to select one of the three LED wavelengths. This turns on the red, green, or blue LED. A *blank* is placed in the cuvette slot. The blank is a cuvette containing the solvent used in the solution being studied, usually distilled water. The blank acts as a control by taking into account the small amount of light absorbed by the solvent and by the walls of the cuvette.

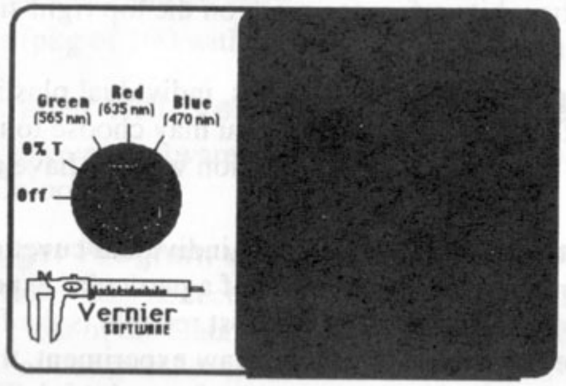


Figure 3

Follow these steps to calibrate the Colorimeter. Specific directions will vary slightly, depending on the program being used.

- The wavelength knob is turned to the "0% T" position. The lid of the cuvette slot must be closed (with or without the "blank" cuvette in place). When the voltage value shown on the computer monitor stabilizes, the user is asked to enter a value. This value will be "0" (for 0% transmittance).

- To do a 100% calibration, the blank is placed in the cuvette slot and the lid of the colorimeter is closed. The wavelength knob of the colorimeter is turned to one of the three LED colors. When the voltage value stabilizes, the user is asked to enter a transmittance value. This value will be "100" (for 100% transmittance).

The colorimeter is now calibrated and you are ready to take absorbance or transmittance readings. These readings can be monitored using any of these programs available from Vernier Software: Data Logger, Voltage Plotter, MPLI Program, or Spectrophotometer Program.

Using Cuvettes with the Colorimeter

The Colorimeter is designed to use polystyrene cuvettes. Fifteen of these cuvettes and lids are supplied with the colorimeter. The cuvettes have a volume of approximately 4 mL. The cuvette slot of the colorimeter is designed to give a snug fit to the cuvette and ensure that it is always in precisely the same position between the LED light source and photodiode. Two opposite sides of the cuvette are ribbed and are not intended to transmit the light from the LED. The two smooth surfaces are intended to transmit light. It is important to position the cuvette correctly in the colorimeter. We recommend this be done as shown in Figure 4, with the ribbed edges facing away from and toward you, and the smooth edges facing left and right. The light travels from left to right from the LED through the cuvette to the photodiode. It should also be noted that there is often a small variation in the amount of light absorbed by the cuvette if it is rotated 180° between trials. To avoid this, you should use a water-proof marker to make a reference mark on the right side of the top edge of the cuvette as shown in Figure 4. Or you can etch a reference mark using the hot tip of a soldering iron. Remind students to align this reference mark with the white reference mark on the top right side of the colorimeter each time they insert a cuvette.

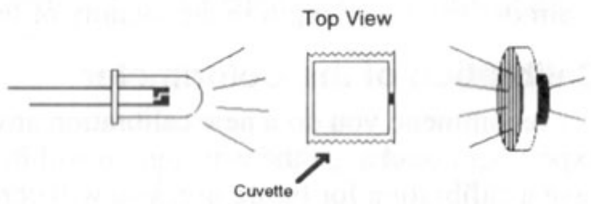


Figure 4

Just like most spectrophotometer sample tubes, individual plastic cuvettes vary slightly in the amount of light they absorb. You may choose to ignore these differences. For most lab exercises, this variation will not have a noticeable effect on experimental results.

For best results, variation in light absorbed by individual cuvettes can be controlled either by using the same cuvette for all trials of a particular experiment or by *matching* a set of cuvettes. The easiest and most reliable is the first method. If a student is going to use five trials for a Beer's law experiment, the five standard solutions can be transferred to the same cuvette for each trial. This requires that the cuvette be clean and dry after each trial *or* rinsed several times with the solution that will be added to it. This method takes very little time and successfully controls a potential variable. It also eliminates concerns over possible scratches that may eventually develop on a cuvette. The effect of the same small scratch is eliminated using the 100% calibration.

As an alternative, you may choose to match cuvettes. Matched cuvettes are a set of cuvettes that all absorb light (when empty) at approximately the same level. This involves more work on the part of the teacher, but saves time in student procedures.

If students have 5 or 6 cuvettes with similar absorbance levels, then each sample can be added to a different cuvette, eliminating the drying or rinsing step described in the previous paragraph. To match a set of cuvettes, first calibrate the colorimeter using the method described in the section on calibration. Use a clean, dry cuvette for the 100% calibration instead of a distilled water blank. Put a reference mark on one of the clear sides of the cuvette so it is always oriented the same way in the cuvette slot. Place each cuvette in the batch in the Colorimeter and record transmittance values for each. When you are finished, group cuvettes according to similar %T values. Each of these groups represents a set of matched cuvettes.

Caps are supplied for the original 15 cuvettes. A cuvette may or may not have a cap on it when placed in the Colorimeter. The purpose of the cap is to prevent evaporation of solvent when an experiment is run over a period of several days. You may find it convenient to store standard solutions in capped cuvettes. If you purchase a replacement set of 100 cuvettes, 20 caps will be included. We felt teachers would probably not need to have one cap per cuvette. The caps can certainly be reused as cuvettes are replaced.

It is very important that solutions be added to a cuvette to the proper depth. Our studies have shown that a "safe level" is between 2.2 and 3.5 mL of solution. When the cuvette is filled to the brim, its total volume is about 4.1 mL. Since the inside diameter of the cuvette is about 1.0 cm x 1.0 cm, this safe level can also be measured on the outside of the cuvette as 2.2–3.5 cm from the inside bottom of the cuvette. These levels are shown in Figure 5. As a reminder to students, these two levels could be marked with a water-proof marker on one of the *ribbed* sides of each cuvette.

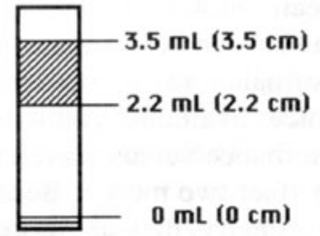


Figure 5

Replacement cuvettes may be purchased using the following ordering code. This package includes 100 cuvettes and 20 caps:

Replacement Cuvettes (pkg of 100 with 20 caps) ..... CUV $10.00

Using the Colorimeter with Vernier Software Products

Here is a summary of Vernier Software interface units and software programs that can be used with the Colorimeter.

Using the Data Logger Program with the Serial Box Interface or the ULI

- Prepare the computer for data collection by opening "Colorimeter" from the Data Logger Experiment Files of the Data Logger program. This file is set up to collect absorbance (or percent transmittance) on the vertical axis and concentration on the horizontal axis (Beer's law). If your version of Data Logger does not have this file, we will be glad to send you a copy. You can also set it up yourself. Choose Event Mode from the Collect Menu. Choose New Column under the Data menu. Click on the pop-up menu at the top-left of the dialog box and change it from Formula to Prompted. Type the name and unit of the concentration in the edit boxes.
- Choose Calibrate from the Collect menu. Then select Calibrate Now. For the first point of the two-point calibration, turn the wavelength knob of the Colorimeter to "0% T" and close the lid (with or without a cuvette in the slot). When the voltage stabilizes, enter "0". For the second point of the two-point calibration, place the

blank cuvette in the cuvette slot and close the lid. Turn the wavelength selection knob to the LED color required for the experiment. When the voltage stabilizes, enter "100".

- Click the Start button. You are now ready to collect absorbance-concentration data.
- Place the cuvette containing the sample solution in the cuvette slot, close the colorimeter lid, and wait for the absorbance to stabilize. When it is stable, click on the Keep button. Enter the concentration value of the solution in the highlighted Concentration box. Continue collecting data in this manner for the remainder of your samples.
- It is also possible to collect *absorbance* (or transmittance) versus *time* using the Colorimeter Data Logger Experiment File. Click on the label of the horizontal axis and select *Time* in place of *Concentration* from the pop-up menu. The graph axes should now be absorbance and time.

Using Spectrophotometer Program (Macintosh) with the Serial Box Interface or the ULI

- Spectrophotometer Program has three experimental modes available to the user: Absorbance versus concentration (Beer's law), absorbance versus time, and absorbance versus wavelength. Since the Colorimeter has only three wavelength choices available, you will not be able to obtain a wavelength spectrum using the absorbance versus wavelength experimental mode. However, you may use either of the other two modes. Because these two modes are designed specifically for colorimetry, they are more convenient and have more options available than Data Logger. In the absorbance versus concentration experimental mode, it is possible to interpolate and determine the concentration of an unknown sample. Calibration is conveniently built into the program. Graphs, data tables, and statistics are easily viewed or printed.

Using the Voltage Plotter Program with the VIU or the MPLI Program with the MPLI

Both of these programs use a similar method for calibrating and monitoring. The calibration is done using percent transmittance. You can also collect percent transmittance data for individual samples. It is not possible to collect absorbance data using these programs. However, absorbance can easily be calculated using a scientific calculator using either of these formulas:

$$A = \log(100/\%T) \text{ or } A = 2 - \log\%T$$

Once % T values have been converted to absorbance, use the Vernier Software Graphical Analysis program (MS-DOS Version) to make a plot of absorbance versus concentration. Here is the general method for calibrating and collecting percent transmittance data using Voltage Plotter or the MPLI program:

- Select Z - Calibration, followed by Z - Calibrate the Active Inputs. For Label and Unit of Input Signal, type in "Transmit" and "%".
- Turn the wavelength knob of the Colorimeter to the "0% T" position and close the lid of the Colorimeter. When the voltage displayed on the monitor stabilizes, enter "0" to represent 0% transmittance.
- Turn the wavelength knob to the desired LED light color. Place a blank cuvette with solvent (usually distilled water) in the cuvette slot. Close the lid of the

Colorimeter. When the voltage displayed on the monitor stabilizes, enter "100" to represent 100% transmittance. The colorimeter is now calibrated.

- Return to the Main Menu and select K - Monitor Input vs. Keyboard. Enter "Conc" and "mol/L" for Label and Unit of the Independent Variable. Place the cuvette containing the sample solution in the cuvette slot. When the percent transmittance is stable, press <Space Bar>, and then enter the concentration value for the sample. Continue with this procedure until you have measured the transmittance of all of your samples. After you have converted the percent transmittance data to absorbance using the formula, A = log(100/%T), you can make a graph of absorbance versus concentration using Graphical Analysis (MS-DOS Version).

Using Spectrophotometer Program (MS-DOS Version) with the VIU or MPLI

- Spectrophotometer Program has three experimental modes available to the user: Absorbance versus concentration (Beer's law), absorbance versus time, and absorbance versus wavelength. Since the Colorimeter has only three wavelength choices available, you will not be able to obtain a wavelength spectrum using the absorbance versus wavelength experimental mode. However, you may use either of the other two modes. Because these two modes are designed specifically for colorimetry, they are more convenient and have more options available than Voltage Plotter or the MPLI Program. For IBM users, this program offers the significant advantage of being able to monitor *absorbance* as well as percent transmittance data. This eliminates the need to independently convert percent transmittance to absorbance using a formula. In the absorbance versus concentration experimental mode, it is possible to interpolate and determine the concentration of an unknown sample. Calibration is conveniently built into the program. Graphs, data tables, and statistics are easily viewed or printed.

Using Voltage Plotter III with the Voltage Input Unit or the MPLI Program with MPLI

Both Voltage Plotter III and MPLI Program use similar methods for calibrating and monitoring. Calibration is done using percent transmittance. You can also collect percent transmittance data for individual samples. It is not possible to collect absorbance data using these programs. However, absorbance can easily be calculated using a scientific calculator using either of these formulas:

$$A = \log(100/\%T) \text{ or } A = 2 - \log\%T$$

Once % T values have been converted to absorbance, you may use the Vernier Software Graphical Analysis III program to make a plot of absorbance versus concentration. Here is the general method for calibrating and collecting percent transmittance data using Voltage Plotter or the MPLI Program.

- Select Z - CALIBRATION, followed by Z - CALIBRATE THE ACTIVE INPUTS. For LABEL and UNIT OF INPUT SIGNAL, type in "TRANSMIT" AND "%".
- Turn the wavelength knob of the Colorimeter to the "0% T" position and close the lid of the Colorimeter. When the voltage displayed on the monitor stabilizes, enter "0" to represent 0% transmittance.
- Turn the wavelength knob to the desired LED light color. Place a blank cuvette with solvent (usually distilled water) in the cuvette slot. Close the lid of the

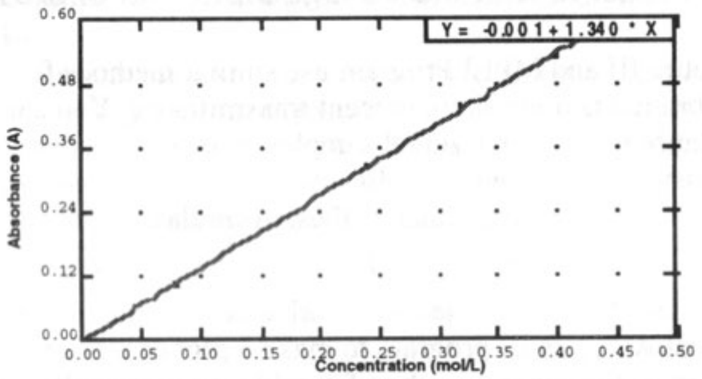
Colorimeter. When the voltage displayed on the monitor stabilizes, enter "100" to represent 100% transmittance. The colorimeter is now calibrated.

- Return to the Main Menu and select K - MONITOR INPUT VS. KEYBOARD. Enter "Conc" and "mol/L" for LABEL and UNIT OF THE INDEPENDENT VARIABLE. Place the cuvette containing the sample solution in the cuvette slot. When the percent transmittance is stable, press <SPACE BAR>, and then enter the concentration value for the sample. Continue with this procedure until you have measured the transmittance of all of your samples. After you have converted the percent transmittance data to absorbance using the formula, A = log(100/%T), you can make a graph of absorbance versus concentration using Graphical Analysis III.

Suggested Experiments

BEER'S LAW

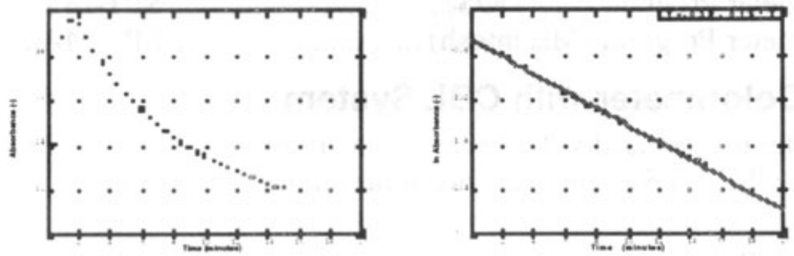
- **Crystal Violet:** Dilute solutions of crystal violet yield a good Beer's law curve using the green LED (565 nm). A stock solution of 8 X 10^{-5} M crystal violet is prepared by adding 65.3 mg of solid crystal violet to enough water to yield 2 liters of solution. Dilute to obtain standard solutions.
- **Copper Sulfate:** Standard solutions that are 0.1, 0.2, 0.3 and 0.4 M $CuSO_4$ will yield a good Beer's law curve at 635 nm (red LED). Or prepare a stock solution by adding 10 g of NH_4NO_3 to 10 mL of 0.1 M $CuSO_4$ and 90 mL of 0.20 M NH_3 (forms the $Cu(NH_3)_4^{2+}$ complex ion) and dilute to obtain standard solutions.
- **$Ni(NO_3)_2$ or $NiSO_4$**: Vernier Software sells the laboratory manual *Chemistry with Computers*. This manual has an experiment, "Determining Concentration of a Solution: Beer's Law." It uses standard and unknown solutions of $Ni(NO_3)_2$, and can easily be used with the Colorimeter. Use the red LED color (635 nm). Data from this lab is shown here using the Data Logger program.



- **Food Coloring Solutions: Red, Blue, Green:** A less expensive alternative to using the solutions above is to prepare solutions using food coloring. We have obtained very good Beer's law curves using these solutions. We added about 6 drops of red, blue or green McCormick brand food coloring to 1 liter of water. The red solution can be analyzed using the blue LED (470 nm), the green solution with the blue LED (470 nm) or the red LED (635 nm), and the blue solution with the red LED (635 nm). Since the actual concentration of the solutions will not be known, refer to the original solution as "100%" and then dilute to 80, 60, 40, and 20%. Check the original solution to see that its absorbance is not greater than 0.550.

DETERMINATION OF THE RATE LAW FOR THE REACTION OF CRYSTAL VIOLET

The data below was determined by reacting 10 mL of 8 x 10^{-5} M crystal violet solution (see Beer's Law above) and 5.0 mL of 0.10 M hydroxide ion in a total volume of 100 mL. The graph on the left is absorbance versus time. The graph shown at the right is the natural log of absorbance versus time, showing the reaction to be first order with respect to crystal violet. The second graph was made using the New Column menu in Data Logger. The new column, with the formula "ln(A)", is selected as the vertical axis for the graph. This graph can also be prepared using Graphical Analysis (Apple II, MS-DOS, or Macintosh version).



ANALYSIS OF CATIONS, ANIONS, WATER HARDNESS OR DISSOLVED OXYGEN USING HACH REAGENT PILLOWS

To determine the concentration of an ion in a colorless solution using a colorimeter, an agent must be added to the solution to yield color (such as a colored complex ion) or turbidity through the formation of a precipitate. The assumption is that the intensity of the color (and its resulting ability to absorb light from the LED) is proportional to the concentration of the ion in solution. Hach Company markets pre-massed *pillows* for analysis of such ions as nitrate (NO_3^-), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), water hardness (Ca^{2+}), or dissolved oxygen. A wavelength and colorimetry method is recommended for each of these ions. Pillows are available for various concentration levels (i.e., 1-100 mg/L, 1-10 mg/L). Try adding these pillows to samples of water collected near your school and analyze for ion concentrations using your Colorimeter. You may obtain a Hach Catalog by writing to: Hach Company, P.O. Box 389, Loveland, CO 80539

DETERMINE THE EQUILIBRIUM CONSTANT, K_C, FOR THE $FeSCN^{2+}$ REACTION

The lab manual, *Chemistry with Computers* contains an experiment for determining the equilibrium constant for this well-known reaction in chemistry. The experiment is listed under the title "Chemical Equilibrium: Finding a Constant, K_C".

TURBIDITY TESTS AND RATES OF SETTLING

Test for turbidity of stream samples using standard methods with the Colorimeter. Devise a method for determining the settling rate of sands of different coarseness.

LIGHTSTICK INVESTIGATIONS

Determine the reaction order, effect of temperature on reaction rate, and activation energy for the chemiluminescent reaction occurring in a light stick. Two experiments in *Chemistry with Computers* are dedicated to this interesting chemical reaction. Turn off the LED light source and monitor the light emitted from the lightstick itself.

MONITORING YEAST GROWTH

Use the Colorimeter to monitor the growth of yeast colonies. Increasing numbers of cells absorb more light from the LED light source.

Ordering Information

Colorimeter (includes 15 cuvettes and lids) ..... COL-DIN $99.00
Replacement Cuvettes (pkg of 100 with 20 lids) ..... CUV $10.00

NOTE: You may use Data Logger, Voltage Plotter or MPLI Program with the Colorimeter. However, either of the Spectrophotometer Programs have additional features that will enhance the use of the Colorimeter with IBM or Macintosh computers.

Spectrophotometer Program (MS-DOS) ..... SPT-IBM $39.95
Spectrophotometer Program (Macintosh) ..... SPT-MAC $39.95

Using the Colorimeter with CBL System

The Colorimeter can be used with the Texas Instruments Calculator-Based Laboratory™ (CBL™) System. Here are some suggestions for using it with the CBL System:

- Connect the CBL unit to the graphing calculator using the I/O ports located on each unit. Make sure to push both plugs in firmly.
- Using a CBL-DIN adapter, connect the sensor to any of the Analog input ports on the top or left side of the CBL unit (CH1, CH2, or CH3). In most cases, CH1 is used. This adapter is available from Vernier Software for $5.
- Run a program to monitor the signal from the sensor. We *strongly recommend* using programs specifically developed for use with this sensor, such as the Vernier Software "CHEMBIO" program.

The Vernier programs for the CBL are available on the Internet or they can be obtained in disk form. Once you have these programs, it is a simple matter to load them into a calculator using TI-GRAPH LINK™ .

- To obtain our programs through the World Wide Web, download the files from our web site:

  www.vernier.com

- The programs can also be obtained on disk. Our Vernier CBL Data Collection Disk contains the programs we have written for all of the TI graphing calculators. An IBM-compatible disk (order code CBL-DP-I) or Macintosh disk (order code CBL-DP-M) is available for $10.

Calibrating with the CBL

The programs we have developed for TI graphing calculators allow you to perform a two-point calibration for sensors like the Colorimeter. When you perform such a calibration, you will place the electrode in two different environments to establish the calibration for the sensor. Here is the general procedure:

1. The calibration process involves two steps. In the first step, close the lid of the Colorimeter, set the knob on the Colorimeter to 0%T, and allow the reading on the CBL to stabilize. Press the [Trigger] button on the CBL and enter 0 when asked to "ENTER REFERENCE:".

2. Now set the knob on the Colorimeter to a wavelength and insert a blank cuvette. Allow the reading on the CBL to stabilize and, again, press the [Trigger] button on the CBL. Enter 100 at the second "ENTER REFERENCE:" prompt.
3. When calibration is complete, the CBL will be set up with the conversion equation, and you can now perform an experiment.