Determining the Concentration of a Solution: Beer's Law

The primary objective of this experiment is to determine the concentration of an unknown copper (II) sulfate solution. The CuSO₄ solution used in this experiment has a blue color, so Colorimeter users will be instructed to use the red LED. Spectrometer users will determine an appropriate wavelength based on the absorbance spectrum of the solution. A higher concentration of the colored solution absorbs more light (and transmits less) than a solution of lower concentration.



Figure 1

You will prepare five copper (II) sulfate solutions of known concentration (standard solutions). Each solution is transferred to a small, rectangular cuvette that is placed into the Colorimeter or Spectrometer. The amount of light that penetrates the solution is used to compute the absorbance of each solution. When you graph absorbance *vs*. concentration for the standard solutions, a direct relationship should result. The direct relationship between absorbance and concentration for a solution is known as *Beer's law*.

You will determine the concentration of an unknown CuSO₄ solution by measuring its absorbance. By locating the absorbance of the unknown on the vertical axis of the graph, the corresponding concentration can be found on the horizontal axis. The concentration of the unknown can also be found using the slope of the Beer's law curve.

OBJECTIVES

- Prepare and test the absorbance of five standard copper (II) sulfate solutions.
- Calculate a standard curve from the test results of the standard solutions.
- Test the absorbance of a copper (II) sulfate solution of unknown molar concentration.
- Calculate the molar concentration of the unknown CuSO₄ solution.

MATERIALS

Vernier computer interface* computer Logger Pro Vernier Colorimeter or Spectrometer one cuvette five 20×150 mm test tubes two 10 mL pipets or graduated cylinders two 100 mL beakers 0.40 M copper (II) sulfate, CuSO₄, solution copper (II) sulfate, CuSO₄, unknown solution pipet pump or pipet bulb distilled water test tube rack stirring rod tissues (preferably lint-free) *No interface is required if using a Spectrometer

PROCEDURE

Both Colorimeter and Spectrometer Users

- 1. Obtain and wear goggles.
- 2. Obtain small volumes of 0.40 M CuSO₄ solution and distilled water in separate beakers. **WARNING**: *Copper (II) sulfate solution*, CuSO₄: *Do not eat or drink when using this product—harmful if swallowed. Causes skin and eye irritation.*
- 3. Label five clean, dry, test tubes 1–5. Use pipets to prepare five standard solutions according to the chart below. Thoroughly mix each solution with a stirring rod. Clean and dry the stirring rod between uses.

| Test Tube | 0.40 M CuSO₄ (mL) | Distilled H₂O (mL) | Concentration (M) |
|-----------|----------------------|-----------------------|----------------------|
| 1 | 2 | 8 | 0.080 |
| 2 | 4 | 6 | 0.16 |
| 3 | 6 | 4 | 0.24 |
| 4 | 8 | 2 | 0.32 |
| 5 | ~10 | 0 | 0.40 |

- 4. Prepare a *blank* by filling a cuvette 3/4 full with distilled water. To correctly use cuvettes, remember:
 - Wipe the outside of each cuvette with a lint-free tissue.
 - Handle cuvettes only by the top edge of the ribbed sides.
 - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
 - Always position the cuvette so the light passes through the clear sides.

Spectrometer Users Only (Colorimeter users proceed to the Colorimeter section)

- 5. Connect the Spectrometer to the computer. Choose New from the File menu.
- To calibrate the Spectrometer, place the blank cuvette into the cuvette slot of the Spectrometer, choose Calibrate ► Spectrometer from the Experiment menu. Wait for the Spectrometer to warm up, and then click OK.
- 7. Determine the optimal wavelength for creating this standard curve.
 - a. Remove the blank cuvette, and place the 0.40 M standard into the cuvette slot.
 - b. Click Collect. The absorbance vs. wavelength spectrum will be displayed. Click Stop.
 - c. To set up the data collection mode and select a wavelength for analysis, click Configure Spectrometer Data Collection, **1**.
 - d. Click Abs *vs*. Concentration (under the Set Collection Mode). Under the list of wavelengths, click Clear Selection. Choose the wavelength nearest to 635 nm from the list. Click OK to continue and proceed to Step 8.

Colorimeter Users Only [Not you]

- 5. Connect a Colorimeter to Channel 1 of the Vernier computer interface. Connect the interface to the computer.
- 6. Start the Logger *Pro* program on your computer. Open the file "17 Colorimeter" from the *Advanced Chemistry with Vernier* folder.
- 7. Calibrate the Colorimeter.
 - a. Place the blank in the cuvette slot of the Colorimeter and close the lid.
 - b. Press the < or > button on the Colorimeter to select the wavelength of 635 nm (Red).
 Press the CAL button until the red LED begins to flash and then release the CAL button.
 When the LED stops flashing, the calibration is complete.

Both Colorimeter and Spectrometer Users

- 8. You are now ready to collect absorbance-concentration data for the five standard solutions.
 - a. Click Collect
 - b. Using the solution in Test Tube 1, rinse the cuvette twice with ~1 mL amounts and then fill it 3/4 full. Wipe the outside with a tissue and place it in the device (Colorimeter or Spectrometer). Close the lid on the Colorimeter.
 - c. After the absorbance readings stabilize, click [™] Keep, type **0.080** in the edit box, and click [™]
 - d. Discard the cuvette contents as directed. Using the solution in Test Tube 2, rinse and fill the cuvette 3/4 full. Wipe the outside and place the cuvette in the device. After the absorbance readings stabilize, click [∞] Keep, type **0.16** in the edit box, and click [∞].
 - e. Repeat the procedure for Test Tubes 3–5. **Note**: Do not test the unknown solution until Step 9.

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- f. When you have finished testing the standard solutions, click **s**top.
- g. Examine the graph of absorbance *vs*. concentration. Click Linear Regression, 🖾. A bestfit linear regression line will be shown for your five data points.
- 9. Write down the absorbance values, for each of the five trials, in your data table.
- 10. Determine the absorbance value of the unknown $CuSO_4$ solution.
 - a. Obtain about 5 mL of the *unknown* CuSO₄ in another clean, dry, test tube. Record the number of the unknown in your data table.
 - b. Rinse the cuvette twice with the unknown solution and fill it about 3/4 full. Wipe the outside of the cuvette, place it into the device. **Important**: The reading in the meter is live, so it is not necessary to click **Collect** to read the absorbance value.
 - c. Read the absorbance value displayed in the meter. When the displayed absorbance value stabilizes, record its value as Trial 6 in your data table.
 - d. Select Interpolate from the Analyze menu. Find the absorbance value that is closest to the absorbance reading you obtained in Step c above. Determine the concentration of your unknown CuSO₄ solution and record the concentration in your data table.
 - e. Dispose of any of the remaining solutions as directed.

| Trial | Concentration (mol/L) | Absorbance |
|-------|--------------------------|------------|
| 1 | 0.080 | |
| 2 | 0.16 | |
| 3 | 0.24 | |
| 4 | 0.32 | |
| 5 | 0.40 | |
| 6 | Unknown number | |

DATA TABLE

DISCUSSION QUESTIONS

- 1. What is the molar concentration of your unknown sample of copper (II) sulfate solution?
- 2. What factors are included in the Beer's law expression for determining how much light passes through a liquid solution?
- 3. How would your test results be affected if you left fingerprints on the sides of the cuvette in line with the light path of the Spectrometer (or colorimeter)?
- 4. Could this method of testing be used to determine the concentration of a NaCl solution?