Determining the Concentration of a Solution: Beer’s Law

The primary objective of this experiment is to determine the concentration of an unknown nickel (II) sulfate solution. You will be using the Colorimeter shown in Figure 1. In this device, red light from the LED light source will pass through the solution and strike a photocell. The NiSO₄ solution used in this experiment has a deep green color. A higher concentration of the colored solution absorbs more light (and transmits less) than a solution of lower concentration. The Colorimeter monitors the light received by the photocell as either an absorbance or a percent transmittance value.

You are to prepare five nickel sulfate solutions of known concentration (standard solutions). Each is transferred to a small, rectangular cuvette that is placed into the Colorimeter. The amount of light that penetrates the solution and strikes the photocell is used to compute the absorbance of each solution. When a graph of absorbance vs. concentration is plotted for the standard solutions, a linear relationship should result. The linear relationship between absorbance and concentration for a solution is known as Beer’s law.

**Beer’s Law Theory**

Beer’s law is useful because by we can determine the concentration of a solution by measuring absorbance, if we have calibrated the colorimeter. To fully understand how Beer’s law works, we can follow how the colorimeter measure absorbance.

**MEASURING LIGHT INTENSITY**

The first thing we measure is how much light shines through pure water (the “blank”). Every measurement we do will be compared to this solution so it make sure it’s well-rinsed! In the diagram, clear water about 4.6 light units come through the clear water.

The second step is to refill the cuvette (a perfectly square test-tube) with our sample and measure the intensity of light that comes through it. In the second diagram, about 3.2 light units come through (as we will see in a moment, the actual units for light intensity do not matter – they will cancel out). In this example, the fraction of light that was transmitted through the sample (relative to water) is:
\[ T = \frac{I_{\text{sample}}}{I_{\text{water}}} = \frac{3.2}{4.6} = 0.696 \approx 0.70 \]

where \( T \) is the fraction transmitted, \( I_{\text{sample}} \) is the light intensity from the second diagram, and \( I_{\text{water}} \) is the light intensity from the first diagram. Notice that \( T \) is a pure fraction – any units would cancel. We could also say that 70% of the light was transmitted. The other 30% of the light was absorbed by the sample atoms / molecules. If we increase the concentration of our sample, there will be more molecules and less light should make it through our sample – in the third diagram, only 1.8 light units come through:

\[ T = \frac{I_{\text{sample}}}{I_{\text{water}}} = \frac{1.8}{4.6} = 0.391 \approx 0.39 \]

In this case 0.39 or 39% of the light came through.

**GETTING A LINEAR (DIRECT) RELATIONSHIP - ABSORBANCE**

If we take the log of the transmittance to get “absorbance,” we will get a linear (direct) relationship between absorbance and transmittance:

\[ A = \log(T) \]

Notice that we actually do minus the log. If you get a strange number for \( T \), you may have taken the log of %\( T \). The linear relationship will now hold:

\[ A \propto C \quad \text{or} \quad A = kC \]

Absorbance ranges from 0.000 for a perfectly clear solution up to 1.0 – 1.5 for a relatively dark solution and above 3 for a really dark solution (like ink). Most instruments cannot measure absorbencies above 3. For good chemistry results, try to keep the absorbance below 1.2.

**BEER’S LAW**

The full equation for Beer’s law is \( A = \varepsilon b C \) where \( A \) is absorbance (no units), \( \varepsilon \) is called the “molar absorbtivity” \( \frac{L}{\text{mol} \cdot \text{cm}} \) or \( \frac{1}{M \cdot \text{cm}} \), \( b \) is the path length (cm), and \( C \) is concentration (mol/L or M). In Beer’s law, the constant is separated into two parts – the path length and molar absorbtivity. Path length is straightforward – if we make our sample thicker, the light has to pass through more sample molecules and more is absorbed.

Most lab experiments use a 1.000 cm cuvette. Molar absorbtivity is more complex and depends on the type of sample (ink and dye solutions are very dark while metal solutions like NiSO\(_4\) are lighter in color) and the wavelength of light.
MEASURING CONCENTRATION

To determine the concentration of an unknown NiSO₄ solution, we need to find the value of ε for our sample at the wavelength use by the colorimeter. While many values are tabulated, it is better to calibrate the colorimeter to correct for instrumental imperfections. To calibrate, we will make several solutions with known concentration, measure their absorbance, and plot the results. If done correctly, this will yield a straight line and we can determine its slope.

Once a good calibration is obtained, any number of unknown solutions can be tested – just measure the absorbance and calculate the concentration (shown graphically). Since the cuvette holds about 3 mL, we are only using a small amount of sample.

THE ALTERNATIVE

If this seems like too much work, another way to find out how much NiSO₄ would be to evaporate the sample and weigh the residual. This process would only take about 1-2 hours of evaporation (watch the sample carefully so it doesn’t boil over) per sample and we’d have to get enough sample to weigh. While 1 liter of 0.4 M NiSO₄ weighs 32 grams, it would take a lot of time and energy to evaporate it. The 3 mL sample size colorimetry uses would only weigh 0.096 g.

Procedure

1. Add about 30 mL of 0.40 M NiSO₄ stock solution to a 100-mL beaker. Add about 30 mL of distilled water to another 100-mL beaker.

2. Prepare 10 mL solutions of 0.08, 0.16, 0.24, and 0.32 from the 0.40 M NiSO₄:
   a. Label four clean, dry, test tubes 1-4 (the fifth solution is the beaker of 0.40 M NiSO₄). Use a pipette to dispense 2, 4, 6, and 8 mL of 0.40 M NiSO₄ solution into Test Tubes 1-4, respectively.
   b. Using a clean pipette to deliver 8, 6, 4, and 2 mL of distilled water into Test Tubes 1-4, respectively.
   c. Thoroughly mix each solution with a stirring rod. Clean and dry the stirring rod between stirrings.
   d. Dispense approximately 10 mL of 0.40 M NiSO₄ into Test Tube 5.

<table>
<thead>
<tr>
<th>#</th>
<th>NiSO₄ (mL)</th>
<th>H₂O (mL)</th>
<th>Conc. (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>5</td>
<td>~10</td>
<td>0</td>
<td>0.40</td>
</tr>
</tbody>
</table>

These solutions are called “standards” or “standard solutions”. They are used to establish a relationship between absorbance and concentration for the colorimeter. Volumes and concentrations for the trials are summarized in the table. Record your visual observations about the solutions.

3. Open the file “Exp 11 Colorimeter” in the Experiment 11 folder of Chemistry with Computers. The vertical axis has absorbance scaled from 0 to 0.6. The horizontal axis has concentration scaled from 0 to 0.5 mol/L.

4. You are now ready to calibrate the Colorimeter. Prepare a blank by filling a cuvette ¾ full with distilled water. To correctly use a Colorimeter cuvette, remember:
• All cuvettes should be wiped clean and dry on the outside with a tissue.
• Handle cuvettes only by the top edge of the ribbed sides.
• All solutions should be free of bubbles.
• Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the Colorimeter.

5. Calibrate the Colorimeter.
• Holding the cuvette by the upper edges, place it in the cuvette slot of the Colorimeter.
• If your Colorimeter has an AUTO CAL button, set the wavelength on the Colorimeter to

First Calibration Point
  i. Choose Calibrate from the Experiment menu and then click Perform Now.
  ii. Turn the wavelength knob on the Colorimeter to the “0% T” position.
  iii. Type “0” in the edit box (next to the active “Keep” button).
  iv. When the displayed voltage reading for Input 1 stabilizes, click Keep.

Second Calibration Point
  i. Turn the knob of the Colorimeter to the Red LED position (635 nm).
  ii. Type “100” in the edit box (next to the active “Keep” button).
  iii. When the displayed voltage reading stabilizes, click Keep, then click OK.

2. You are now ready to collect absorbance data for the five standard solutions. Click Collect. Empty the water from the cuvette. 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 Colorimeter. After closing the lid, wait for the absorbance value displayed on the monitor to stabilize. Then click Keep, type “0.080” in the edit box, and press the ENTER key. The data pair you just collected should now be plotted on the graph.

3. Discard the cuvette contents into a waste beaker. Rinse the cuvette twice with the Test Tube 2 solution, 0.16 M NiSO₄, and fill the cuvette ¾ full. Wipe the outside, place it in the Colorimeter, and close the lid. When the absorbance value stabilizes, click Keep, type “0.16” in the edit box, and press the ENTER key.

4. Repeat the Step 3 procedure to save and plot the absorbance and concentration values of the solutions in Test Tube 3 (0.24 M) and Test Tube 4 (0.32 M), as well as the stock 0.40 M NiSO₄ (The unknown is measured later). When you have finished with the 0.40 M NiSO₄ solution, click Stop.

5. In your laboratory notebook, record the absorbance and concentration data pairs that are displayed in the Table window.

6. Examine the graph of Absorbance vs. Concentration (the “Calibration Curve”). To see if the curve represents a direct relationship between these two variables, click the Linear Regression button. A best-fit linear regression line will be shown for your five data points. This line should pass near or through the data points and the origin of the graph.

7. Obtain an unknown NiSO₄ solution by taking your cuvette to the stock room. Each person will do their own unknown, even if you are working in a group. Wipe the outside of the cuvette, place it into the Colorimeter, and close the lid. Read the absorbance value displayed in the Meter window. (Important: The reading in the Meter window is live, so it is not necessary to click Collect to read the absorbance value.) When the displayed absorbance value stabilizes, record its value in Trial 6 of the Data and Calculations table. Repeat for the other person’s unknown.
Processing the Data

1. Use the following method to determine the unknown concentration.
   - With the linear regression curve still displayed on your graph, choose Interpolate from the Analyze menu. A vertical cursor now appears on the graph. The cursor’s x and y coordinates are displayed at the bottom of the floating box (x is concentration and y is absorbance).
   - Move the cursor along the regression line until the absorbance (y) value is approximately the same as the absorbance value you recorded in Step 7.
   - The corresponding x value is the concentration of the unknown solution, in mol/L.
2. Click on the graph title (“Absorbance vs. Concentration”). Add your names to the title in the edit box and click OK.
3. Print the graph of absorbance vs. concentration, with a regression line and interpolated unknown concentration displayed. You will need to print it again for your lab partner.

Chemical Waste Disposal

Nickel ion is toxic and should never be poured down the drain. Collect your NiSO\(_4\) waste in a beaker during the experiment then pour it into the waste container provided on the center island.

Report

Submit a report with the standard format for this experiment. Your calculations are limited to determination of the diluted standard concentrations. Be sure to report the concentration of your assigned unknown (each partner gets one unknown).
1. Preparation of standard solutions (Step 2) involves dilution – remember the dilution equation, \( M_1V_1 = M_2V_2 \). Show how you would use this equation to make each solution in the table.

Example (solution #1):

| \( M_1 \) | 0.40 M |
| \( V_1 \) | ? |
| \( M_2 \) | 0.08 M |
| \( V_2 \) | 10.00 mL |

\[ M_1V_1 = M_2V_2 \]
\[ V_1 = \frac{M_2V_2}{M_1} = \frac{(0.08 \, M)(10.00 \, mL)}{(0.40 \, M)} = 2.00 \, mL \]

To make the solution, use 2.00 mL of the 0.40 M NiSO\(_4\) solution and add enough water to make a total of 10.00 mL (need 8.00 mL).

Solution #2:

| \( M_1 \) | 0.40 M |
| \( V_1 \) | ? |
| \( M_2 \) | 0.16 M |
| \( V_2 \) | 10.00 mL |

Solution #3:

| \( M_1 \) | 0.40 M |
| \( V_1 \) | ? |
| \( M_2 \) | 0.24 M |
| \( V_2 \) | 10.00 mL |

Solution #4:

| \( M_1 \) | 0.40 M |
| \( V_1 \) | ? |
| \( M_2 \) | 0.32 M |
| \( V_2 \) | 10.00 mL |

Solution #5:

| \( M_1 \) | 0.40 M |
| \( V_1 \) | ? |
| \( M_2 \) | 0.40 M |
| \( V_2 \) | 10.00 mL |

2. A lazy chemist only makes one standard solution and gets the following results:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 M (Water)</td>
<td>0.000</td>
</tr>
<tr>
<td>0.40 M</td>
<td>0.442</td>
</tr>
</tbody>
</table>

a. What is the molar absorbtivity (\( \varepsilon \)) if he used a standard 1.000 cm cuvette? \textbf{Be sure to report the correct units.}

b. What is the concentration of an unknown solution with an absorbance of 0.234?