Spectroscopy Experiment

Light interacts with matter. The study of this interaction is called spectroscopy. Much of the information about the nature of matter and atomic electron configurations has been determined by spectroscopic methods.

Light is electromagnetic radiation, a form of energy that moves at the speed of light. The energy of light is dependent on its wavelength and frequency. Common types of spectroscopy are infrared IR, ultra-violet UV, and visible spectroscopy. In this experiment the absorption of light will be measured in the visible portion of the electromagnetic spectrum.

Substances that have visible color absorb some portion of the visible region of the electromagnetic spectrum. Chlorophyll appears green because it is absorbing red light (at about 655 nm) and violet light (at about 430 nm), while transmitting or reflecting the yellow, blue and green wavelengths. The light at 655 nm and 430 nm absorbed by chlorophyll provides the energy for photosynthesis. The green color of chlorophyll is a qualitative measurement of the substance.

Many metal ions such as aluminum, chromium, iron, and cobalt can be analyzed by visible spectroscopy. The metal ion is extracted from the sample and prepared for analysis. Today we will be determining the absorbance spectrum for the cobalt (II) ion and determining the concentration of cobalt (II) chloride in unknown samples.

Cobalt (II) chloride is often used to detect moisture. The anhydrous form of cobalt chloride is blue, while the hydrated form cobalt (II) chloride hexahydrate is pink. It is sometimes used as invisible ink. Using an eyedropper, drop several drops of the cobalt chloride solution on a paper towel. Observe the color as the paper towel dries. Did the cobalt chloride change colors?

Cobalt can be found in batteries, alloys, drill bits and machine tools, dyes and pigments as cobalt blue, magnets, and tires. Cobalt is also a component of vitamin B12, an essential vitamin.

The concentration of a substance will influence the degree of absorption; therefore, the measurement of light absorbed can be used to determine the concentration of a substance. Beer's Law states that absorbance A is proportional to concentration c in the equation: \( A = k c \). Graphing absorbance vs. concentration should yield a straight line with a slope of k. An absorbance spectrum and a standard calibration line (or Beer's Law plot) is shown in the sample data section below.

Objectives:

1. Calibrate and use a UV-VIS spectrophotometer to measure absorbance.
2. Measure the absorption spectrum of two different substances in the visible range. (Absorbance vs. wavelength)
3. Prepare several dilutions from a known standard solution.
4. Calculate the concentration of each dilution from $M_1V_1 = M_2V_2$.
5. Determine the standard calibration line or Beer’s law plot (absorbance vs. concentration) for the dilutions.
6. Determine the concentration of an unknown solution.
7. Investigate the flame emission spectrum of several metal ions in aqueous solutions.

**Chemicals:**

Aqueous stock solutions of known concentration of two different ions: $\text{Co}^{2+}$ and $\text{FeSCN}^{2+}$ preferred. [If these are not available $\text{Ni}^{2+}$, $\text{Cr}^{2+}$, $\text{MnO}_4^-$, OR $\text{Cr}_2\text{O}_7^{2-}$ can be substituted.]

For flame spectroscopy: $\text{BaCl}_2$, $\text{SrCl}_2$, $\text{CaCl}_2$, $\text{CuCl}_2$, $\text{LiCl}$, $\text{KCl}$, and $\text{NaCl}$ solutions

Burets of .150 M $\text{CoCl}_2$ and R.O.$\text{H}_2\text{O}$ supplied by stockroom

**Equipment:**

Cuvettes
Spectrophotometer
Small test tubes
Test tube rack
Wooden coffee stirrers
Ignitor
Bunsen burner

Prior to attending lab, read the sections of our textbook dealing with spectrophotometry (in Chapter 4); and wavelength, frequency, and energy in (Chapter 6).
Sample Data Analysis
The absorbance spectrum of an aqueous solution of chromium III ion, Cr\(^{3+}\) was taken at nine different wavelengths, from 400 to 600 nm. The spectrum was determined for two solutions of different concentrations. Below is a table of the data, followed by the absorbance spectrum.

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Absorbance of 0.03 M Cr(^{3+})</th>
<th>Absorbance of 0.01 M Cr(^{3+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>0.356</td>
<td>0.139</td>
</tr>
<tr>
<td>425</td>
<td>0.388</td>
<td>0.125</td>
</tr>
<tr>
<td>450</td>
<td>0.297</td>
<td>0.102</td>
</tr>
<tr>
<td>475</td>
<td>0.152</td>
<td>0.057</td>
</tr>
<tr>
<td>500</td>
<td>0.161</td>
<td>0.052</td>
</tr>
<tr>
<td>525</td>
<td>0.263</td>
<td>0.104</td>
</tr>
<tr>
<td>550</td>
<td>0.402</td>
<td>0.147</td>
</tr>
<tr>
<td>575</td>
<td>0.518</td>
<td>0.157</td>
</tr>
<tr>
<td>600</td>
<td>0.488</td>
<td>0.154</td>
</tr>
</tbody>
</table>

Wavelength of Maximum Absorbance
Lambda Max = 575 nm

A maximum in absorbance occurs at 575 nm. This wavelength is used to determine the relationship between absorbance and concentration.
The graph of absorbance versus concentration at a constant wavelength is called a standard calibration line or "Beer's law plot". The standard solutions are prepared as dilutions from a concentrated solution of known concentration. The concentration of each dilute solution is calculated from \( M_{cVc} = M_{dVd} \) or \( M_1V_1 = M_2V_2 \).

Following is a data table and a standard calibration line.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Absorbance</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.077</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>0.139</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.307</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.446</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.614</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.768</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The data in the table above was plotted below. The graph "fits" the data to the equation of a straight line. It uses the format, \( y = mx + b \). In this equation the slope is the value of \( m \). The slope has units of absorbance per molarity, or liters per mole (inverse of molarity).

Beer's law \( A = k \ c \) relates absorbance to concentration.

\( A \) is absorbance measured with the spectrophotometer. Absorbance is a unitless value, \( k \) is the slope of the line, when absorbance is plotted versus concentration, and \( c \) represents concentration in moles per liter.

For the graph above, the Beer's law equation \( A = k \ c \) would be:

\[ A = 15.38 \ c \]
The concentration of an unknown solution can be found by rearranging the equation:
\[ c = \frac{A}{k} = \frac{A}{15.38} \]
If an unknown solution has an absorbance of .412, its concentration would be calculated as:
\[ c = \frac{.412}{15.38} = .0268 \text{ moles/liter} \]

This experiment contains several parts. Each part has instructions and a worksheet to enter collected data and make calculations.

**Calibrating or Standardizing the Genesys 20 spectrophotometer**
The spectrophotometer must be calibrated with a water blank before using it and each time the wavelength is changed.

**Determination of the absorption spectrum.**
The absorbance of two different metal ions will be measured at different wavelengths in the visible spectrum.

**A standard calibration line or Beer's law plot of absorbance versus concentration**
Several dilutions will be prepared from a solution of known concentration of one of the metal ions. The concentration of the diluted solutions will be calculated from the known concentration of the standard solution, using the equation \( McVc = MdVd \) or \( M_1V_1 = M_2V_2 \).

The absorbance of each diluted solution will be measured at the wavelength of maximum absorbance. Absorbance will be graphed against concentration to determine the slope of the line.

**Determination of the concentration of an unknown**
The absorbance of an unknown solution will be measured. Concentration will be calculated using information obtained from the standard calibration line.

**Flame emission spectrum**
The flame emission spectrum of several metal ions will be studied.
Download Data Spreadsheet.

Calibrating or Standardizing the Genesys 20 spectrophotometer:

Plug the spectrophotometer in and turn it ON with the switch in the back. Allow 10 minutes for it to warm up.

The spectrophotometer must be calibrated before using it and every time the wavelength is changed.

1. Set the wavelength to 400 nm. Using the nm (up-button) or nm (down-button).
2. Add R.O. water to a cuvette. Clean any excess liquid from the exterior with a Kimwipe. This cuvette is called the blank.
3. Place the blank in the sample compartment with the line facing the ridge on the container, and close the lid.
4. Set the instrument to 0% absorbance with 0 ABS 100% T button.
5. The instrument is now set for absorbance measurements at 400 nm.
6. Each time the wavelength is changed, recalibrate the spectrophotometer with the water blank by repeating Steps 2 - 5 at the desired wavelength.

Determination of the absorbance spectrum for aqueous metal ions:

1. Standardize the spectrophotometer (see above directions.)
2. Obtain enough solution of cobalt (II) chloride CoCl₂ of known concentration to fill a cuvette to just below the label (about 7 mL).
3. Fill another cuvette with a solution of iron (III) thiocyanate FeSCN₂⁺ solution.
4. Measure the absorbance of the two solutions at wavelengths from 400 to 600 nm, in 25 nm increments. Each time the wavelength is changed, the spectrophotometer must be standardized with the water blank (see above directions).
5. Record the absorbance values in the Excel spreadsheet. The computer will graph the absorption spectrum from the entered data. Determine the wavelength of maximum absorbance for each solution. Why are the wavelengths of maximum absorbance different? Notice the color of the CoCl₂ solution compared to the FeSCN₂⁺ solution.

Determination of the standard calibration line (or Beer’s Law plot) from absorbance and concentration data:

** Only CoCl₂ solutions will be used in this section.

1. Set the spectrophotometer at the wavelength of maximum absorbance and standardize it again with the water blank.
2. A buret containing the cobalt (II) chloride standard solution and another buret containing R.O. water will be set up in the lab.
3. With the provided burets carefully prepare additional dilutions. (See the suggested volumes below. The cuvette holds approximately 6 to 7 ml.) Label and place the cuvettes in a test tube rack.

<table>
<thead>
<tr>
<th>Amount CoCl₂</th>
<th>Amount Water</th>
<th>Total Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ml</td>
<td>7 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>1 ml</td>
<td>6 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>2 ml</td>
<td>5 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>3.5 ml</td>
<td>3.5 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>5 ml</td>
<td>2 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>6 ml</td>
<td>1 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>7 ml</td>
<td>0 ml</td>
<td>7 ml</td>
</tr>
</tbody>
</table>

4. Before measuring the absorbance, mix the dilutions well by covering with aluminum foil and inverting each cuvette.

5. Hold the cuvette up to the light and look for smudges, fingerprints, and air bubbles. Both will diffract the light and give poor results. Wipe any fingerprints off the cuvette with Kimwipes. Dislodge air bubbles with a disposable pipet or clean stirring rod.

6. Measure the absorbance for each dilution and record the data in the data sheet.

7. Calculate the concentration from the dilution of the known standard using \( M_1V_1 = M_2V_2 \). The concentration of the known standard is on the bottle. Record the calculated concentrations on the data sheet.

8. Determine the slope of the line of absorbance versus concentration. This may be done easily by following the steps written on the Excel spread sheet or with a graphing calculator.

**Determination of the concentration an unknown**

1. Measure the absorbance of the unknown at the maximum wavelength. If the value is below 1.000 (or within the range of your calibration line), continue.

**However, if the value of the absorbance is greater than 1.000,** dilute the solution with R.O. water until its absorbance is below 1.000. Record the volumes used in the dilution. Suggestion: 3 mL unknown mixed with 3 mL H₂O = 6 mL total solution.

2. Use the equation \( A = k c \), the measured absorbance \( A \), and the slope of the line \( k \) to calculate \( c \), the concentration of the unknown solution.

Note: After calculating the concentration \( c \), use \( M_1V_1 = M_2V_2 \) to account for any dilutions made in step 1.
Flame Emission Spectroscopy

1. Light the Bunsen burner.
2. Soak a wooden splint in one of the solutions provided.
3. Wave the end of the wooden splint through the center of the burner’s flame.
4. Observe, describe, and record the color of the flame.
5. Place the burnt splint in a beaker of water. Do NOT put any splints in the wastebasket. Your instructor will collect all wooden splints at the end of the experiment.
6. Repeat the above steps with each of the different solutions.
7. Use the spectrum of visible light in your textbook in Chapter 6, to determine the approximate wavelength for the color of light that is emitted.
   (Note: Sodium is often present as a contaminant. Its yellow color interferes with identification of some elements.)