Absorbance Spectroscopy and Beer's Law

- Learn about the Interaction of Photons with Molecules and Ions in Solution.
- Learn about Spectroscopy.
- Learn about Beer’s Law.

In this laboratory exercise, we will probe the behavior of electrons within molecules and ions using Absorbance Spectroscopy. In particular, we will examine the absorbance spectrum of Cupric Ions in an aqueous environment in the visible region of the electromagnetic spectrum. We will leverage the concentration dependence of the absorbance of a solution of Cupric Ions to determine the concentration of a solution of unknown concentration. This is a very common analytic technique for determining the concentration of absorbing species.

Previously we have observed the emission spectrum of Hydrogen and Helium atoms; where the excited atoms relax and emit photons of energy equivalent to the atom's quantum state transition. Typical electronic transitions within atoms and molecules are such that the corresponding photons have energies in the Visible and Ultraviolet (UV-VIS) regions of the spectrum.

Likewise, if a photon of the correct energy impinges on an atom, molecule or ion, such that this energy matches the energy required for a quantum state transition, the photon can be absorbed:
In these cases, the Energy difference between the participating quantum states is related to the Energy of the photons via:

\[ \Delta E = E_{\text{photon}} = \frac{hc}{\lambda}. \]  

(Eq. 1)

Thus, the photons absorbed or emitted by a sample of the atom, molecule or ion in question are a direct probe of the energy difference between quantum states of that atom, molecule or ion.

A major complication occurs if an absorbing or emitting species is in a condensed phase; a liquid or a liquid solution. If the absorbing molecule/atom is in a solution, it is surrounded by constantly jostling solvent molecules. Thus, each molecule/atom finds itself in a slightly different environment than its brothers. This causes the energy gap between the quantum states responsible for the absorbance of photons to be slightly different for each molecule/atom. This means we will have a series of very, very closely spaced absorbance lines. Practically, this means the Absorbance Spectrum will be a broad band, rather than a sharp line. This is as diagramed below:

In this case, we usually identify the absorbance band by the wavelength of maximal absorbance, \( \lambda_{\text{max}} \).

Measuring the wavelength of absorbed or emitted photons is accomplished in a spectrometer. The spectrometer will also measure the Intensity of the absorbance or emission. This intensity is then related to the number, or concentration, of the absorbing/emitting species.
A Vis Spectrometer consists of a White Light source, dispersive optics to separate the wavelengths of the light, a sample compartment and a detector.

Light from the source passes through an Entrance Slit and is focused on the dispersive element, such as a diffraction grating. This separates out the various wavelengths comprising the White Light into a rainbow of colors. The desired wavelength is selected by rotating the dispersive element such that the desired color passes through an Exit Slit, through the Sample and then onto a detector.

The Transmittance of the light is then defined as:

\[ T = \frac{P}{P_0} \]  
(Eq. 2)

This is simply a measure of how many photons pass through the sample without being absorbed. This is then related to the Absorbance by:

\[ A = -\log T \]  
(Eq. 3)
As shown schematically above, the Absorbance will depend directly on the Concentration \( c \) of the absorbing species. Two other factors determine the Absorbance of a sample. The further light must travel through a sample, the so-called pathlength \( b \), the greater is the Absorbance. The ability of the absorbing species to absorb light at the given wavelength, known as the extinction coefficient \( \varepsilon \), a quantum mechanical effect, will also determine the Absorbance. The relationship between these influences and the Absorbance is given by the Beer-Lambert Law; after Johann Heinrich Lambert and August Beer, co-discoverers of this relationship.

\[
A = \varepsilon b c \quad \text{(Eq. 4)}
\]

Note the Absorbance is directly proportional to each factor. However, this proportionality has its limitations.

Beer's law describes the absorption behavior only of dilute solutions and in this sense is a limiting law. At concentrations exceeding about 0.01 M, the average distance between ions or molecules of the absorbing species are diminished to the point where each particle affects the charge distribution, and thus the extent of absorption, of its neighbors. Because the extent of interaction depends on concentration, the occurrence of this phenomenon causes deviations from the linear relationship between absorbance and concentration. A similar effect sometimes occurs in dilute solutions of absorbers that contain high concentrations of other species, particularly electrolytes. When ions are very close to one another, the molar absorptivity of the analyte can be altered because of electrostatic interactions, and this can lead to departure's from Beer's law.

The Beer-Lambert Law is the basis of using absorbance measurements to determine the concentration of absorbing analytes in solution. To analyze for a given analyte, \( \varepsilon \) is first determined using a series of standard solutions of known \( c \). (Typically \( \lambda_{\text{max}} \) is chosen as the wavelength for measuring the absorbance values.) Then the absorbance of the analyte solution is determined. The unknown concentration is then determined via:

\[
c = \frac{A}{\varepsilon b} \quad \text{(Eq. 5)}
\]

Alternatively a calibration curve is constructed and used directly to determine the analyte’s concentration. A series of standard solutions containing the analyte at known concentrations are measured. Then, the least squares method can be used to determine the “best fit” line to a plot of absorbance vs. concentration. This calibration curve is the used to determine the analyte concentration in an unknown solution whose absorbance has been measured.
One last point regarding absorbance spectroscopy needs to be addressed; different colored solutions absorb different wavelengths of light. The color of the light absorbed is directly related to the color of the light transmitted; i.e., the color we see. The color absorbed is the Complement of the color we see:

<table>
<thead>
<tr>
<th>Color Absorbed</th>
<th>Wavelength (nm)</th>
<th>Color Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>650-780</td>
<td>Bluish-Green</td>
</tr>
<tr>
<td>Orange</td>
<td>595-650</td>
<td>Greenish-Blue</td>
</tr>
<tr>
<td>Yellow</td>
<td>580-595</td>
<td>Blue</td>
</tr>
<tr>
<td>Yellowish-Green</td>
<td>560-580</td>
<td>Violet</td>
</tr>
<tr>
<td>Green</td>
<td>500-560</td>
<td>Purple</td>
</tr>
<tr>
<td>Bluish-Green</td>
<td>490-500</td>
<td>Red</td>
</tr>
<tr>
<td>Greenish-Blue</td>
<td>480-490</td>
<td>Orange</td>
</tr>
<tr>
<td>Blue</td>
<td>435-480</td>
<td>Yellow</td>
</tr>
<tr>
<td>Violet</td>
<td>380-435</td>
<td>Yellowish-Green</td>
</tr>
</tbody>
</table>

This simply means our spectrometer must be set to a wavelength corresponding to the maximal absorbance of the absorbing species.

In this experiment, we will measure the absorbance of a solution of Cupric Ions (Cu^{2+}) using a visible absorbance spectrometer; either the Cole Parmer S2150UV (right image) or the Unico 2800 (left image).
By doing this for a series of solutions of known concentration, we can determine the value of the Extinction Coefficient, $\varepsilon$. Subsequently, an absorbance measurement of a solution of unknown concentration can be used to determine its concentration.
Pre-Lab Safety Questions

Some general safety questions this week.

1. What is the ANSI standard for the flow rate of an eyewash and a safety shower?

2. Should a chemical safety shower have a floor drain? Explain.

3. What is the ANSI standard temperature for an eyewash's Water supply?

4. According to the ANSI document Z358.1, what is the maximum distance allowable from your workstation to a eyewash station or an emergency shower?
Procedure

1. Obtain an appropriate amount of solid Cupric Sulfate Pentahydrate, \( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} \), to prepare 50mL of a 0.1M solution. You should weigh this out on an analytical balance. Record the actual mass you use. (Consult with your instructor to confirm your calculations.)

2. Use a 50mL volumetric flask to prepare this solution.

3. Now, fill a spectrometer cuvette. (Handle the cuvette from the edges to avoid getting your finger prints on the faces. Use a Kimwipe to clean the faces of the cuvettes before making any readings.)

4. Now, prepare 50 mL of a 1:2 dilution of your above Cupric Sulfate solution. Fill another spectrometer cuvette.

5. Prepare additional 1:2 dilutions until you have a total of 5 filled cuvettes of decreasing concentration.

6. Obtain 10 mL of a solution of Cupric Sulfate of unknown concentration. Fill another cuvette with this solution of unknown concentration.

7. Proceed to the instrument room to make the absorbance measurements.

8. Use the UV-VIS spectrometer to measure the absorbance of each solution at 660nm, the \( \lambda_{\text{max}} \) for the Cupric Sulfate Pentahydrate solution. Use a Water filled cuvette as a “blank” to first “zero” the instrument. (Your lab instructor will assist you in making these measurements. These spectrometers are scanning, double-beam instruments. We will be using them to make single wavelength measurements, so will only be using a fraction of its capabilities.)
**Data Analysis**

1. Using a software package such as *Excel*, prepare an Absorbance calibration curve using the Cupric Sulfate solutions of known concentration. To this plot add a **Trendline** and obtain the equation of this line.

2. Using its measured Absorbance and your calibration curve’s **Trendline** equation, determine the concentration of the unknown Cupric Sulfate solution.

3. Determine the Molar Absorptivity $\varepsilon$ using the Beer-Lambert Law; (Eq. 4).
Post Lab Questions

1. In your spectrophotometer Absorbance measurements, how do you expect the errors in subsequent measurements to compare to the error in the measurement for the stock solution?

2. What is the color of the Cupric Sulfate solution used in the Absorbance measurements? What color light is being absorbed by these solutions?

3. Many times analytes only absorb weakly in the UV-VIS region of the spectrum, creating a bit of an experimental challenge. However, in many instances, when complexed with so-called "colorimetric agents", they will produce a measurable spectrum. Bi\textsuperscript{3+} complexes with Thiourea to form a colored complex which Absorbs light at $\lambda_{\text{max}} = 470$ nm with an Extinction Coefficient of $\varepsilon = 9320 \text{ M}^{-1}\text{cm}^{-1}$. What is the Concentration of the complex in a solution that has a measured Absorbance $A = 0.265$ in 1.00 cm cell.

4. If a solution's absorbance is too high, greater than ~4-5, it is best to dilute the system and re-measure the absorbance. This is because typical spectrometers have difficulty measuring high absorbance values accurately. Why is this? (Hint: Think in terms of the number of photons reaching the detector.)