Reading assignment:.    http://en.wikipedia.org/wiki/Beer-Lambert_law

**Goals**

We will study the spectral properties of a transition metal-containing compound.  We will also study the relationship between the concentration and absorbance for transition metal-containing compounds.

**Materials and Supplies**

Spectronic 301 spectrophotometer, 6-8 1 cm cuvettes, 100 mL volumetric flask, 10 mL graduated cylinder, 10 mL transfer pipet, 4-6 test tubes,  ~2 g solid cobalt(II) chloride salt or solid copper(II) sulfate.

**Discussion**

Why do food colorings have particular colors?  The answer to this question involves the discussion of photons, energy, and energy levels in atoms and molecules.  Solutions often possess color because they absorb photons at wavelengths within the range of visible light (400-700 nm).  In the process of absorption the energy of the absorbed photon is converted into electronic energy in the atom or molecule that absorbed the photon.  The study of the absorption of light by atoms or molecules and its dependence on wavelength or frequency is called spectrophotometry.

The energy of a photon is related to its wavelength ($\lambda$) and its frequency ($\nu$):

$$E_{photon} = \frac{hc}{\lambda} = h\nu$$

where E is the energy of the photon, h is Planck's constant $(6.626 \times 10^{-34}\text{ J}\times\text{s})$,  and c is the speed of light in a vacuum (2.998 x $10^8$ m/s).  The frequency of a photon is perceived by humans as color.  For example, photons with a frequency of 6.8 x $10^{14}$ $s^{-1}$ are perceived as violet.

$$E_{photon} = (6.626 \times 10^{-34}\text{ J}\times\text{s})(6.8 \times 10^{14}\ s^{-1}) = 4.5 \times 10^{-19}\text{J}$$

A frequency of 6.8 x $10^{14}$ $s^{-1}$ corresponds to a wavelength of 4.4 x $10^{-7}$ m when the speed of the photon is equal to the speed of light in a vacuum (c = $\lambda\nu$).  Using the wavelength of the photon gives the same result for the energy of the photon:

$$E_{photon} = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34}\text{ J}\times\text{s})(2.998 \times 10^8\frac{m}{s})}{(4.4 \times 10^{-7}\text{m})} = 4.51 \times 10^{-19}\text{J}$$

The absorption of a photon by an atom or molecule can be represented using energy level diagrams.  Consider the absorption of a photon by a hydrogen atom at 434.0 nm.  This corresponds to the absorption of a photon that possesses the energy difference between n = 3 and n = 2.

When a visible wavelength photon is absorbed by an atom or a molecule, an electron is promoted to an electronic orbital of higher energy.  This increases the energy of the atom or molecule.  We can take advantage of this phenomenon to gain information about the molecular structure of the molecule and its energy states since the energy of the photon is equal to the difference between two energy levels of the atom or molecule.  For example, a solution of cobalt(II)

**Energy levels of hydrogen atom**
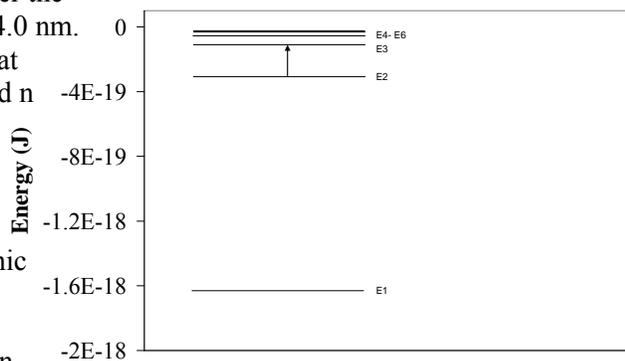
chloride in water has a reddish tint to it.  Visible light consists of a wavelength range of 400-700 nm where 1 nm = $10^{-9}$ m. This range corresponds to colors from violet to red:

| wavelength $\lambda$ (nm) | frequency $\upsilon$ (s$^{-1}$) | color |
|---|---|---|
| 420 | 7.1 x $10^{14}$ | violet |
| 470 | 6.4 x $10^{14}$ | blue |
| 530 | 5.7 x $10^{14}$ | green |
| 580 | 5.2 x $10^{14}$ | yellow |
| 620 | 4.8 x $10^{14}$ | orange |
| 700 | 4.3 x $10^{14}$ | red |

**Complementary colors**
The color wheel shows the relationship between colors of absorbed light and colors of light that we see.  If a color is absorbed by a sample then we see the complementary color.  The complementary color is the color on the opposite side of the wheel.  For example, if white light is shined on a sample and red light is absorbed then the sample is perceived as green by humans.



white light
(all colors)

green light
observed

object absorbs
red light
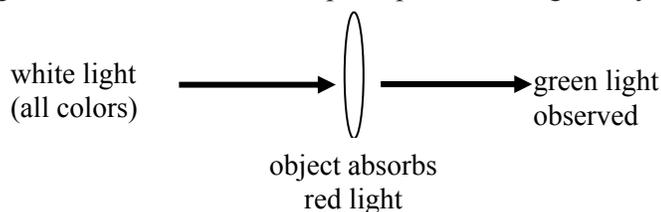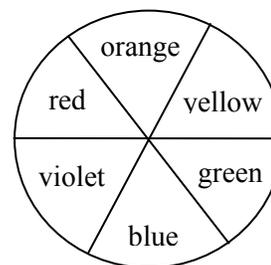
**Transition metal complexes**
Many compounds possessing transition metal ions absorb visible light when dissolved in water.  For example, copper(II) sulfate appears as a blue solution in water.  Nickel(II) chloride produces a green solution in water.  The reddish tint of the cobalt(II) chloride solution results from the absorbance of light that is blue-green.  Once the blue-green light has been removed from the white light, the perception of color results from the light that was not absorbed.  If we were to shine white light on a sample of cobalt(II) chloride in water we would find that light is absorbed over a range of about 450-600 nm.  This broad range of wavelengths actually corresponds to several colors.  But most of the absorbance occurs near 520 nm, in the blue-green part of the spectrum.

Because different molecules absorb light in different wavelength ranges we can make determinations of what type of molecule is in solution based on the wavelengths of light absorbed.

The absorption of light by transition metals can be described through d-orbitals.  When compounds containing metal ions like copper(II) or cobalt(II) are dissolved in water the water molecules hydrate the metal ions.  The energies of the d-orbitals in the metal ion are affected by the closest water waters around the metal ion. Normally, all 3d orbitals possess the same energy (degeneracy).  But in water this is not the case:

**Cu atom**                                        **Cu$^{+2}$(aq)**

$\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{xy}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{xz}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{yz}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{x2-y2}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{z2}$        $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{x2-y2}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{z2}$

$\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{xy}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{xz}$   $\overline{\phantom{x}}\,\overline{\phantom{x}}$ 3d$_{yz}$
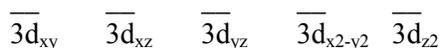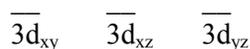
In the copper(II) ion there is an energy difference between the 3d orbitals. If an electron occupies one of the lower energy levels and a photon possessing energy that matches the difference in energy between the lower and upper levels then absorption can take place.

**Spectrophotometers**

A common experiment is to use analytical instrumentation that measures the intensity and wavelength of light. These measurements are made using an instrument called a spectrophotometer. Some spectrophotometers are designed to operate in the ultraviolet and visible region of light. They are often referred to as UV-visible spectrophotometers. Others absorb in the infrared region and are called IR spectrophotometers. A diagram for a spectrophotometer is shown to the right.

a. Light is generated by a **lamp source**.

b. This light is collimated by the use of small openings called **slits**.

c. The collimated light is focused with a **lens**.

d. The **grating** selects individual wavelengths of the white light.

e. The light is then passed through a sample holder called a **cuvette**.

f. The light not absorbed by the sample passes through a **filter** to ensure that light from undesirable wavelengths do not reach the detector

g. The **detector** (phototube) converts light to electrical energy and sends this signal to an amplifier/controller/readout.

h. Data is typically represented as absorbance (or percentage transmittance) and wavelength using a **display**.

(c) lens

(b) slits

(a) light source

(f) filter

(g) detector

(e) cuvette

| Wavelength | Absorbance |
|---|---|
| 460 nm | 0.205 |

(h) display
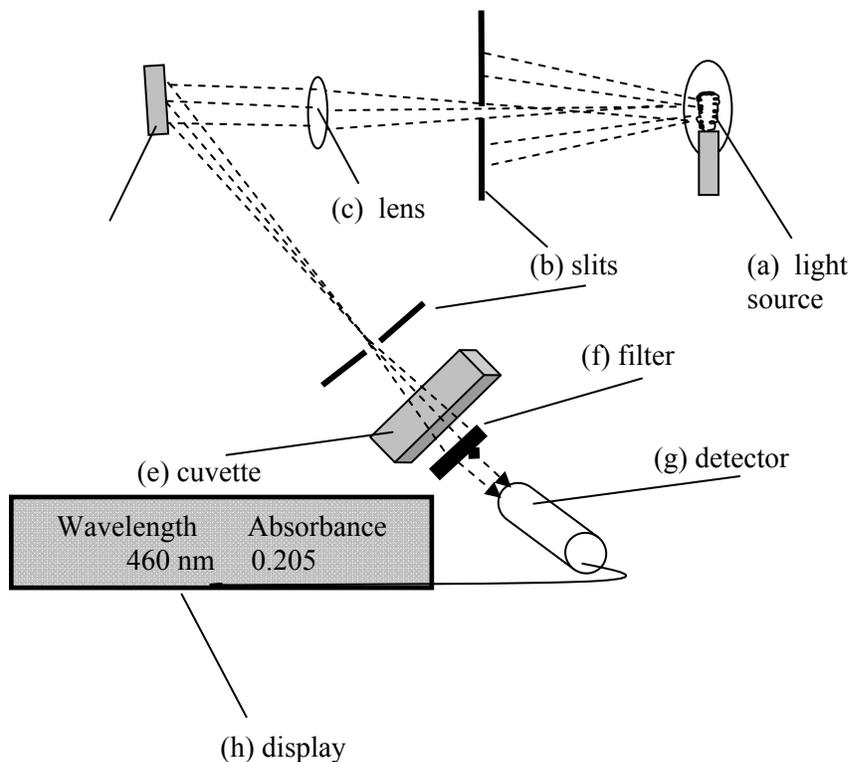
Data from a spectrophotometer can be collected and graphed in what is called an absorption spectrum. An example absorption spectrum is shown below for cobalt(II) chloride in water. A quantity called absorbance (unitless) is plotted on the y–axis and wavelength (in nanometers) is plotted on the x-axis. The maximum absorbance is at a wavelength of 510 nm. This corresponds to a color of blue-green.

The absorption spectrum shown is generated by measuring the amount of light absorption at different wavelengths.

Absorbance is essentially of a measure of the proportion of light striking the sample is being absorbed by the sample. Consider a solution that absorbs visible light.

Absorption spectrum of cobalt (II) chloride

The amount of light that is absorbed by the solution depends on the concentration of the absorbing species in the solution. The intensity of the light shined on the sample is greater than the intensity of light that exits the cuvette if the sample absorbs any of the light. So increasing the concentration of the absorber will result in more photons being absorbed. To imagine an absorbance measurement consider the figure shown below. Light is passed through a sample in a cuvette. The amount of light is measured in terms of intensity (I). The incident intensity is the amount of light shined on the sample and is given the symbol $I_0$. Let's assume that some of the light is absorbed by the sample. The amount of light that exits the cuvette is given the symbol I. I is generally smaller than $I_0$ becuase some of the light is absorbed by the sample. I should never be greater than $I_0$. A measure of the proportion of light that passes through the sample (not absorbed) is **percentage transmittance** (%T):
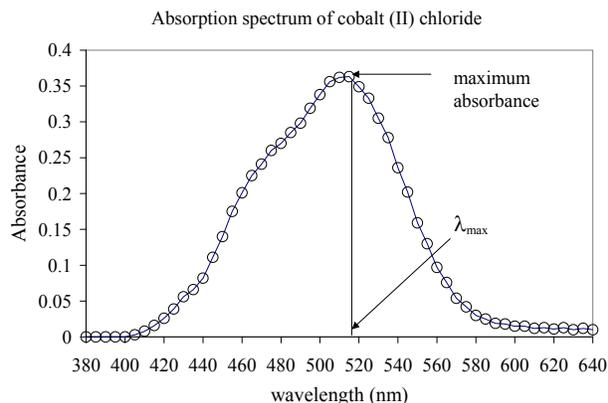
$$\%T = \frac{I}{I_0} \times 100\%$$

Percentage transmittance is a unitless quantity because it's a ratio. Another measure of the amount of light absorbed is **absorbance**. Absorbance is related to percentage transmittance:

$$A = \log\left(\frac{I_0}{I}\right)$$

Note that absorbance is, like percentage transmittance, a unitless quantity because it's a ratio. It is also a logarithmic quantity. Consider the measurements shown in the table below. Let's pass 100 units of light into the sample ($I_0$).

$I_0$: Intensity of light shined on the cuvette
Assume 100 units

I: Intensity of light exiting cuvette
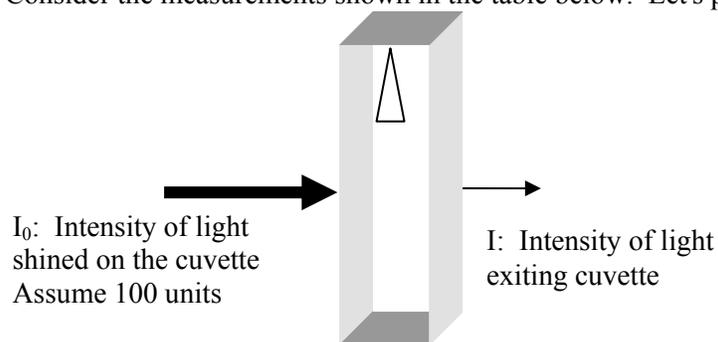
| $I_0$ | $I$ | $\dfrac{I}{I_0}$ | %T | Absorbance |
|---|---|---|---|---|
| | | | $\dfrac{I}{I_0}\text{x}100\%$ | $\log\left(\dfrac{I_0}{I}\right)$ |
| 100 | 100 | 1 | 100 | 0 |
| 100 | 50 | 0.5 | 50 | 0.3 |
| 100 | 10 | 0.1 | 10 | 1.0 |
| 100 | 5 | 0.05 | 5 | 1.30 |
| 100 | 1 | 0.01 | 1 | 2 |
| 100 | 0.1 | 0.001 | 0.1 | 3 |

The absorbance increases as the amount of light that exits the cuvette decreases. The absorbance depends on the concentration of the molecules that absorb the light. This observation can be shown in mathematical form in what is called the Beer–Lambert law (equation 1):

$$\log\frac{I_0}{I}=\varepsilon lc \qquad \text{equation 1}$$

where $\varepsilon$ is the molar absorption coefficient, a constant for a given substance at a given wavelength, c is the concentration of the solution in the units of moles/liter, l is the length of the light path through the solution, $I_0$ is the intensity of the incident light, and I is the intensity of the transmitted light.

Cuvettes are generally 1.0 cm wide which means that l = 1.0 cm. The value of $\varepsilon$ depends on the substance being measured and can have values up to about 100,000 $M^{-1}{\cdot}cm^{-1}$.

The Beer-Lambert law can be simplified to:

$$\log\frac{I_0}{I}=kc \qquad \text{equation 2}$$

where k = $\varepsilon$l. Finally, the quantity $\log\dfrac{I_0}{I}$ is called the absorbance and is given the symbol A. This results in the more common form of the Beer–Lambert Law:

A = kc          equation 3

The important result of equation 3 is that the absorbance is directly proportional to the molar concentration where molar concentration is in the units of $\dfrac{\text{moles}}{\text{liter}}$. Thus, as the concentration of the colored substance increases, less light gets through (i.e., I gets smaller) and A becomes larger (because more light is absorbed). For a known substance the concentration can be found by measuring its absorbance.

According to equation (3), a plot of absorbance vs. concentration at one specific wavelength should give a straight line plot (A is proportional to c). This should enable us to determine the concentrations of unknown solutions of this substance using values of absorbance we have measured.

<div style="text-align: right"><strong>Procedure</strong></div>

**SAFETY PRECAUTIONS**
Safety glasses must be worn during this experiment.

Students work in pairs but measure separate unknowns and prepare separate reports.
Specific instructions describing the use of the Spectronic 301 spectrophotometer are given in the introduction to this manual.

**Part 1**:  **The Absorbance Spectrum of a Transition Metal in Water**
1.  Prepare or obtain a sample of metal hydrate dissolved in water.  This solution is called the **stock** solution.  The stock solution can be prepared by dissolving 1.74 g of the metal hydrate salt in distilled or deionized water using a 100 mL volumetric flask.  If the stock solution has already been prepared then obtain 25 mL of the solution in a clean dry 100 mL beaker.

2.  Before measuring a sample, the spectrophotometer must first measure the absorbance of a blank solution.   A blank solution is one that contains all of the components that the sample possesses, with the exception of the analyte.  For this experiment, the analyte is the metal hydrate complex.  So water will serve as the blank.  Using a transfer pipet, place distilled or deionized water into a 1 cm cuvette, dropwise, until it is about 2/3 full.  Place the plastic lid on the cuvette.  Place the cuvette with the blank solution in the sample holder of the spectrophotometer.  The clear sides of the cuvette should be facing toward you.  The frosted sides of the cuvette scatter light and should be oriented to your left and right.

3.  Close the lid of the spectrophotometer and set the wavelength of the spectrophotometer to 420 nm.

4.  Set the absorbance of the spectrophotometer to zero.  This may take a few seconds.

5.  Open the spectrophotometer lid and place the cuvette with the stock solution into the sample holder and record the absorbance value in the data sheet.

6.  Repeat steps 2-4 for a wavelength of 440 nm.

7.  Continue with measurements every 20 nm up to 680 nm.  Steps 2-4 must be repeated each time the wavelength is changed.

8.  Once you have completed measurements from 420-680 nm identify from the data set which measurement showed the largest absorbance.

9.  Measure the absorbance every 5 nm near the wavelength of maximum absorbance.  For example, if the wavelength of maximum absorbance was found to be 500 nm measure the absorbance at 485, 490, 495, 505, 510, and 515 nm.  Record these measurements in the data sheet.

10.  Review all of your absorbance data.  Identify which wavelength resulted in the largest absorbance value.  The wavelength at which this occurred is often referred to as $\lambda_{max}$.  Record this wavelength and set the spectrophotometer to this wavelength for Part 2.

**Part 2**: **Beer–Lambert  Law**
1.  Prepare diluted solutions from the stock solution.  Use the 20-25 mL of stock solution from Part 1. Dilute using distilled or deionized water.  The solutions are:

2. 2 mL of stock solution diluted to 10 mL.
4. 4 mL of stock solution diluted to 10 mL.
6. 6 mL of stock solution diluted to 10 mL.
8. 8 mL of stock solution diluted to 10 mL.

2. To prepare the first dilution (2), use a transfer pipet to transfer 2.0 mL of the stock solution into a clean, dry 10 mL graduated cylinder. Use a dropper to add distilled or deionized water to the graduated cylinder to the 10.0 mL mark.

2. Transfer this solution to a test tube and label the test tube "2 mL."

3. Repeat steps 2 and 3 with 4 mL, 6 mL, and 8 mL of stock solution.

4. Set the wavelength of the spectrophotometer to $\lambda_{max}$ from Part 1. Then set the absorbance to 0.00 using distilled or deionized water as a blank.

5. Measure the absorbance of each solution prepared in steps 1-3 at $\lambda_{max}$. Record the absorbance for each solution in the data sheet.

**Part 3: Determining the concentration of an unknown sample**
1. You will now be given a cuvette containing a solution of the metal complex you have been working with. Record the unknown number in the data sheet. Measure the absorbance of these solution and record the value in the data sheet.

2. Solutions that you prepared can be rinsed down the sink. Any solutions that were prepared for you should be returned to the instructor.

3. Turn off the spectrophotometer.

**Part 1**.

1.  Use graphical analysis to produce a graph of absorbance (y) versus wavelength (x).

2.  Label the axes and title the graph accordingly. Use all data.

3.  Print a copy for each student in your group.

**Part 2**.

1.  Use graphical analysis to generate a graph of absorbance versus concentration of the metal hydrate solutions.

2.  Concentration needs to be converted to the units of moles per liter from grams per liter.

3.  The concentration of the diluted solutions can be found from the stock solution and the volumes used to prepare them.  For each solution:

a.  Calculate the number of moles of solute in the volume of stock used.

b.  Calculate the molarity from the number of moles and total volume.

The blank should have a concentration of zero mol/L.

4.  The graph should appear linear since absorbance is proportional to concentration for the solutions we measured.

Absorption spectrum of cobalt (II) chloride



Cobalt (II) chloride:  Effect of concentration



$y = 6.728x + 0.0081$
correlation $= 0.9958$

5.  Perform a linear regression analysis of the data (line of best fit).

6.  Use the slope and y-intercept from the line of best fit to calculate the concentration of the unknown solutions.
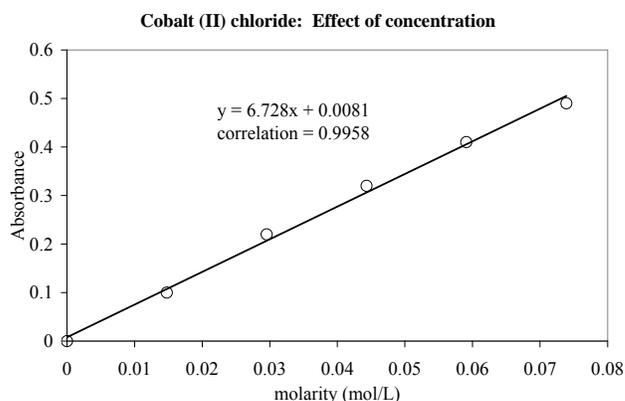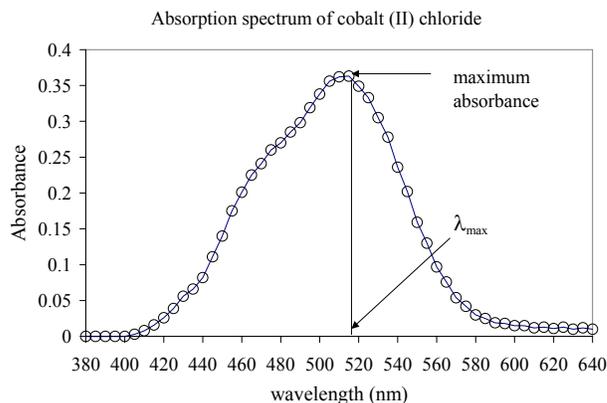
Date _____

Name_____

Partner's Name_____

**Part 1:  The Absorbance of Cobalt(II) Chloride Hexahydrate or Copper(II) Sulfate**

**Measurements at 20 nm intervals**

| $\lambda$ (nm) | absorbance | $\lambda$ (nm) | absorbance |
|---|---|---|---|
| 420 | _____ | 560 | _____ |
| 440 | _____ | 580 | _____ |
| 460 | _____ | 600 | _____ |
| 480 | _____ | 620 | _____ |
| 500 | _____ | 640 | _____ |
| 520 | _____ | 660 | _____ |
| 540 | _____ | 680 | _____ |

**Measurements at 5 nm intervals**

| $\lambda$ (nm) | absorbance |
|---|---|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

Wavelength of maximum absorbance ($\lambda_{max}$)*        _____

Absorbance at this wavelength        _____

*Use this wavelength for all measurements in Part 2.

**Part 2**: **Beer–Lambert Law**

Chemical formula of solution used        (copper or cobalt)        _____

**Data**
Concentration of stock solution  _____ (g/L)

Molar mass of compound        _____ (g/mol)

**Calculations**
Molarity of stock solution        _____ (mol/L)
        Use the molar mass and the concentration in g/L to find the molarity

2.  Dilutions of stock solution

| Solution | Volume of Stock (mL) | Total Volume (mL) | Molarity (mol/L) | Absorbance |
|----------|----------------------|-------------------|------------------|------------|
| blank    |                      |                   |                  |            |
| 2        |                      |                   |                  |            |
| 4        |                      |                   |                  |            |
| 6        |                      |                   |                  |            |
| 8        |                      |                   |                  |            |
| stock    |                      |                   |                  |            |
| Unknown  |                      |                   |                  |            |