Spectrophotometry and the Beer-Lambert Law:
An Important Analytical Technique in Chemistry

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Introduction:

In the last lab you observed that thermal or electrical excitation of atoms caused electrons to be promoted to higher lying energy levels. Subsequent relaxation of the excited electrons to their ground state energy levels was accompanied by emission of photons in the visible region of the electromagnetic spectrum. The distinctive colors emitted during this relaxation process can be used in some cases to identify the element responsible for the emission spectrum.

Over the next two lab periods you are going to turn this phenomenon around and utilize the absorption spectrum of a transition metal ion in aqueous solution. Transition metal ions are known for the wide array of colors they display. In fact, many transition metal ions are responsible for the colored pigments in paints, the color of gemstones like rubies or sapphires, and the colors in stained glass.

Absorption spectra of chemical species (atoms, molecules, or ions) are generated when a beam of electromagnetic energy (i.e. light) is passed through a sample, and the chemical species absorbs a portion of the photons of electromagnetic energy passing through the sample. A classic example of this phenomenon is responsible for our perception of color. Consider the situation where a beam of white light (i.e. sunlight) passes through a sample solution containing chlorophyll (the compound responsible for the color of leaves). The chlorophyll molecules absorb only a few select photons in the blue and red regions of the visible portion of the electromagnetic spectrum. The energies of these absorbed photons cause electrons in the chlorophyll molecule to be excited, and in the plant cell the energy of these excited electrons is used to drive the conversion of carbon dioxide and water to glucose. More important for our purposes is the fact that when the red and blue photons that chlorophyll absorbs are subtracted from white light, the resulting beam of light leaving the solution appears green to our eye, and this is why leaves appear green to us.

If we could measure the total number of photons of all colors that enter the sample and compare that with the total number of photons of all colors that leave the sample, we would find fewer photons exiting the sample than entering the sample. This is consistent with the fact that the chlorophyll molecules absorbed some of the photons from the beam of white light that entered the solution.

A spectrophotometer is an instrument designed to make this measurement. Using some very well understood electronics, this device effectively “counts” the number of photons that enters a sample and compares it with the number of photons that exits a sample. In addition, the instrument is able to take white light and separate it into its constituent colors (i.e. somewhat like
a prism), allowing the user to examine the absorption of light of individual wavelengths with nearly 1 nm resolution.

In optics, that portion of physics that deals with the properties of light, the measurement of the number of photons delivered at a point in a given unit of time is called the Intensity, \( I \). (Higher intensity could be thought of as “brighter” and lower intensity could be thought of as “dimmer”; hence high intensity light will be bright and low intensity light will be dim.) If we measure the intensity of the beam of light entering our sample \( I_o \) and compare it with the intensity of the beam of light exiting our sample \( I \) we can take the ratio \( I/I_o \) to get an indication of what fraction of the light entering the sample was found exiting the sample. This ratio is called the Transmittance:

\[
T = \frac{I}{I_o}
\]

We can convert this ratio into a percentage by multiplying by 100 to get Percent Transmittance (%T):

\[
\% \text{ Transmittance: } \%T = \frac{I}{I_o} \times 100
\]

Thus if the intensity of the light exiting our sample is 76 and the intensity of the light entering our sample is 100, then the Transmittance would be 0.76 and the % Transmittance would be 76%, indicating that 76% of the photons entering our sample are finding their way out of our sample.

For our purposes it is mathematically convenient to define a new concept, Absorbance (A):

\[
\text{Absorbance: } A = -\log_{10} \frac{I}{I_o}
\]

*Absorbance is a direct measure of how much light is absorbed by our sample.* If you play with the formula in your calculator you will find that absorbance can take on values between 0 (at 100% Transmittance) and about 2.0 (at 1% Transmittance); thus large values of absorbance are associated with very little light passing entirely through the sample and small values of absorbance (i.e. those approaching 0) are associated with most of the light passing entirely through the sample.

**The Beer-Lambert Law:**

Consider a solution of a chemical species that absorbs light of a particular wavelength. We could imagine two interesting situations. First, if we pass a beam of light of the appropriate wavelength through a fairly dilute solution, we could imagine that the photons will encounter a small number of the absorbing chemical species, so we might expect a high % transmittance and a low absorbance. Alternatively, if we pass the same beam of light through a highly concentrated solution, we could imagine that the photons will encounter a large number of the
absorbing chemical species, and we might expect a low % transmittance and a high absorbance. Thus absorbance is proportional to the concentration of the sample. Secondly, we could imagine that if we allow the beam of light to encounter the solution for a long period of time we might expect to see a low % transmittance and a high absorbance; whereas, if the beam were allowed to encounter the solution for a short period of time we might expect to see a high % transmittance and a low absorbance. Since light travels at a constant speed, $c = 3.0 \times 10^8 \text{ m/s}$, this implies that the absorbance should also be proportional to the path length of the beam through the sample. These two considerations allow us to establish the following proportionality:

$$A \propto k \times l \times c,$$

where, $k = \text{proportionality constant}$,

$l = \text{path length}$, and

$c = \text{concentration of the absorbing chemical species}$

When the path length is measured in centimeters, and the concentration of the absorbing species is measured in Molarity, the proportionality constant is called the Molar Absorptivity, $\varepsilon$ having units of M$^{-1}$cm$^{-1}$, and our proportionality reduces to the

**Beer-Lambert Law:**

$$A = \varepsilon \times l \times c$$

This technique is used not only by chemists but by scientists of many fields. The Beer-Lambert law allows you, the scientist, to measure the absorbance of a particular sample and to deduce the concentration of the solution from that measurement! In effect, you can measure the concentration of a particular chemical species in a solution as long as you know the species absorbs light of a particular wavelength.

**The Problem:**

Unfortunately, in order to determine concentration of a species in solution, you need to know two key pieces of information:

1) At what wavelength does the chemical species of interest absorb electromagnetic radiation, and

2) What is the value of the molar absorptivity; $\varepsilon$?

**During the first lab** period of this experiment, you will be asked to do the following;

- Make 100 ml aqueous solution of one of these salts; CoCl$_2$.6H$_2$O or NiCl$_2$.6H$_2$O (do not prepare both solutions, ask your instructor for only one salt). Prepare a solution having concentration between 0.05 and 0.25M.
- Use the Spectronic-20D spectrophotometer (see attached appendix for operation instructions) to record the UV-visible absorbance spectrum of the prepared solution between 400 and 950nm. For best absorbance, record the absorbance every 10 nm between 400 and 950 nm.
Use Microsoft Excel on your station computer to record the absorbance of your solution at regular intervals. In the region where absorbance is particularly high, it is strongly suggested that you record absorbance at relatively small intervals (say every 5 nm) in order to accurately determine the wavelength at which maximum absorbance occurs \((\lambda_{\text{max}})\).

Plot the spectrum with absorbance on the y-axis (dependent variable) and wavelength in nm on the x-axis (independent variable, or the one you control) and determine \((\lambda_{\text{max}})\) to be used in next lab period.

Print two copies of the spectrum plot one for each partner.

**During the second lab period** of this experiment you will determine the answer to two questions:

1. What is the value of the molar extinction coefficient or molar absorptivity, \(\varepsilon\), for the metal ion you used in week one.
2. What is the concentration of a transition metal ion in an unknown solution provided by your instructor? The answer to the first question will allow you to answer the second question.

In order to determine \(\varepsilon\) for your metal ion, you will need to construct a calibration curve by first preparing a series of four solutions of the ion. We suggest that you keep your concentrations of metal ion between 0.05 and 0.25M. (If you err on one side or the other of this range, we would suggest that you tend higher instead of lower.) Since you only need a small sample of each solution, say 10-15 mL, it might make sense for you to use the concept of serial dilution to prepare these solutions. Make 100 mL of the most concentrated solution you need from the range of concentrations given (stock solution). For the next most concentrated solution (solution #2), use the first solution as a stock solution, and utilize the dilution formula to calculate what volume of the first solution you need in order to make the desired quantity of solution #2. Make sure you show your instructor your calculations prior to proceeding. Then use solution #2 as a stock solution to prepare a less concentrated; solution #3 the same way. Repeat one more time using solution #3 as a stock solution to prepare solution #4. (Refer to the end of chapter 6 of the McMurry-Fay textbook to review the dilution process.)

At every stage, be sure to record all masses and/or volumes so that you can calculate accurate concentrations of the metal ion in all four solutions. Accurate values here are critical, as they will determine the accuracy of the concentration measurement you make on the unknown solution. Report your calculations in detail.

Once the solutions are prepared, record the absorbance for all four solutions at a single, appropriate wavelength. To choose an appropriate wavelength, examine the absorbance spectrum that you created last lab, and ask yourself, “What wavelength will exhibit the greatest sensitivity to changes in concentration (i.e. what wavelength will show the greatest change in absorbance for a given change in concentration)?” Go to your last data plot of wavelength vs. absorbance and determine the proper wavelength to be used.
Once the absorbance of each of the four solutions is known, use Microsoft Excel to plot absorbance on the y-axis and concentration on the x-axis. Examining the mathematical form of the Beer-Lambert Law, the molar extinction coefficient or molar absorptivity, $\varepsilon$, is a constant for a given transition metal ion, and the path length ($l$) is a constant as long as the same test tube or cuvette is used to make each absorbance measurement. In effect, when $\varepsilon$ is multiplied by $l$, the result is a constant, and the only difference that can give rise to a change in absorbance is the concentration of the absorbing species. Thus, the Beer-Lambert Law establishes a linear relationship between absorbance and concentration:

$$A = \varepsilon \times l \times c$$

$$A = k \times c$$

$$y = mx + b$$

This indicates that absorbance changes proportionally with concentration, making absorbance the dependent variable and concentration the independent variable. If you plot absorbance versus concentration (in molarity) you should obtain a straight-line with a slope equal to the molar extinction coefficient multiplied by the path length. Using a ruler, measure the path length by measuring the inner diameter of the test tube or cuvette you used to make your measurements. Divide the slope of your line by the measured path length (in cm) to calculate the molar extinction coefficient of your transition metal ion. The graph of absorbance versus concentration is called a calibration curve, and it is used to correlate absorbance with the molar concentration of the absorbing species (the transition metal ion in this experiment).

Finally, obtain a sample of an unknown concentration of the transition metal ion you have been working with ($\text{Co}^{2+}$, or $\text{Ni}^{2+}$) from your instructor. Measure and record the absorbance of this solution. Once the absorbance is measured, determine the concentration of the transition metal ion in the unknown. You have two methods available to make this determination. First, you can use your calibration curve to determine concentration by reading what concentration should give the absorbance reading you measured. (You may have to extrapolate your calibration curve beyond the measured values you recorded. If so, please state that you did so in your lab report.) Secondly, you can calculate the concentration algebraically by substituting (1) the measured absorbance, (2) the value of the molar extinction coefficient, $\varepsilon$, that you calculated from your calibration curve and (3) the measured path length (in cm) of the test tube you used to measure the absorbance of the unknown sample into the Beer-Lambert Law. After determining the concentration of your transition metal ion using both methods, compare and state which method you think is more accurate.
Critical Data to Include in your Lab Report:

From Week 1:

- Specify which compound was used
- Show all calculations and accurately describe how you prepared your solution of CoCl$_2$.6H$_2$O or NiCl$_2$.6H$_2$O
- Succinctly describe how $\lambda_{\text{max}}$ was determined
- Include a figure of your absorption spectrum from 400-950 nm and report $\lambda_{\text{max}}$

From Week 2:

- Show all calculations and accurately describe how you prepared the solutions of the chosen transition metal ion used to construct the calibration curve
- Succinctly describe the procedure for constructing the calibration curve
- Include a graph of your calibration curve that includes the linear fit and equation
- Report the value of $\varepsilon$ for your metal ion in units of M$^{-1}$cm$^{-1}$ and show calculations
- Report the measured concentration of the transition metal ion in the unknown solution given to you by your lab instructor. Be sure to include the concentration from both techniques that you used to determine the unknown concentration.
- Discussion comparing the two concentration values obtained and which one you think is more accurate/reliable
- References.