UTA-809
Spectrophotometric Determination of Purity and Concentration

Objective: To determine the purity of the coordination complex, $[Fe(phen)_3]Cl_2$ by determining its molar extinction coefficient (ε). The molar extinction coefficient will also be used to determine the concentration of some solutions of $[Fe(phen)_3]Cl_2$ in which the concentration is unknown.

INTRODUCTION

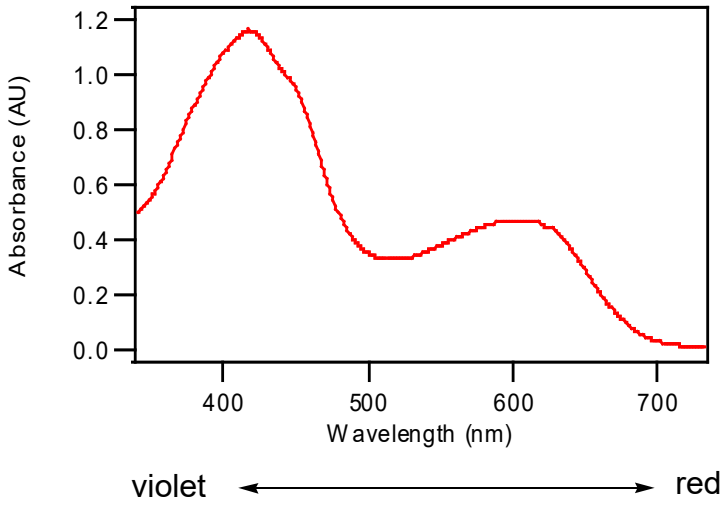
Many chemical compounds are colored and therefore absorb visible light. White light is what we observe when all the colors of the rainbow strike our eyes simultaneously. Recall that the colors of the rainbow appear in the following order: **r**ed, **o**range, **y**ellow, **g**reen, **b**lue, **i**ndigo, **v**iolet. The moniker, ROYGBIV, is a useful way of remembering this order. When an object is colored, it absorbs photons of some colors and reflects photons of other colors. The reflected photons are seen by our eyes. For liquids, only the photons that are **transmitted** through a solution of a compound are observed by our eyes. For example, a blueish substance is one that is absorbing the red-orange-yellow-green photons of white light, leaving only the blue-indigo-violet to be reflected or transmitted to our eyes.

Because there are so many hues and variations in color and not all of us perceive color exactly the same (for example – color blind people), scientists have developed instruments that can record the absorption spectrum of a dilute solution in a reproducible and quantitative manner. A UV-Visible Spectrophotometer (UV-Vis) is the primary instrument of this type and it records the intensity of light at a particular wavelength (λ) and creates a plot of the absorbance of a given sample versus the wavelength (λ). A typical UV-Vis instrument can measure light in the **ultraviolet** (UV), and the **visible** portion of the electromagnetic spectrum. The UV has wavelengths which range from 190 nm to 400 nm and the visible ranges from 400 nm (violet) to 720 nm (red). UV light is not visible to our eyes but is the type of radiation that is responsible for sunburns.

A typical UV-Vis spectrum for compound X is shown in Figure 1. Only the visible portion of the spectrum is shown (360 nm to 720 nm) and it is clear that at least two major absorbance peaks are observed for this compound. The largest peak is observed at 415 nm and the second largest peak at 610 nm. The dip between these two peaks is at ~510 nm which corresponds to green light. Not

surprisingly, compound X appears dark green to our eyes as most of the indigo-violet-blue light is absorbed by the peak around 415 nm and the yellow-orange light by the peak at 610 nm. The resulting green and red light that passes through the sample combines to look dark green to our eyes. *NOTE: Our eyes are not equally good at detecting all colors. The human eye is most sensitive to green thus partially explaining why the sample appears green even though more red photons (light with wavelength ~700 nm) may be reaching our eyes than green ones (~ 510 nm).*

FIGURE 1:



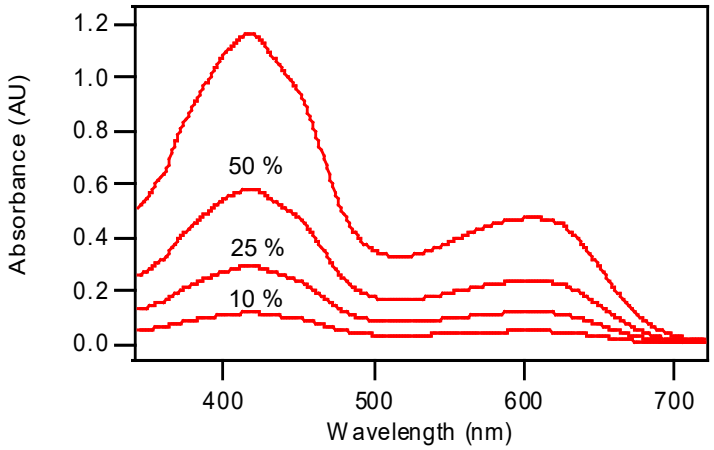
Importantly, the UV-vis spectrophotometer can be calibrated such that it will always record the same spectrum for the same sample (under the same conditions). This means that we can now talk about the color of a compound in a more quantitative way. Instead of saying we have a dark green compound; we can say the compound has absorption maximums (λ_{max}) at 415 and 610 nm.

We can also relate the amount of light absorbed to the intensity of the peak (the absorbance). As shown in Figure 2, when the solution of compound X is diluted to 50 % the original concentration, the absorbance drops by half. Similarly, dilution to 25% and 10% of the original concentration give absorbance values decreased by 75 % and 90 %, respectively. The linear relationship between concentration and the absorbance at a particular wavelength is known as Beer's Law, which is stated mathematically in Equation 1:

$$\mathbf{A = \varepsilon bc} \qquad (1)$$

where **A** = absorbance as measured by a spectrophotometer, **ε** is the molar extinction coefficient (units L/mol·cm), **b** is the cell pathlength (in cm) and **c** is the concentration of the species being examined (mol/L).

FIGURE 2:



For most spectrophotometers and in most experiments we use a 1 cm cuvette as the cell therefore **b** =1 cm (typically) so the expression simplifies to:

$$\mathbf{A = \varepsilon c} \qquad (2)$$

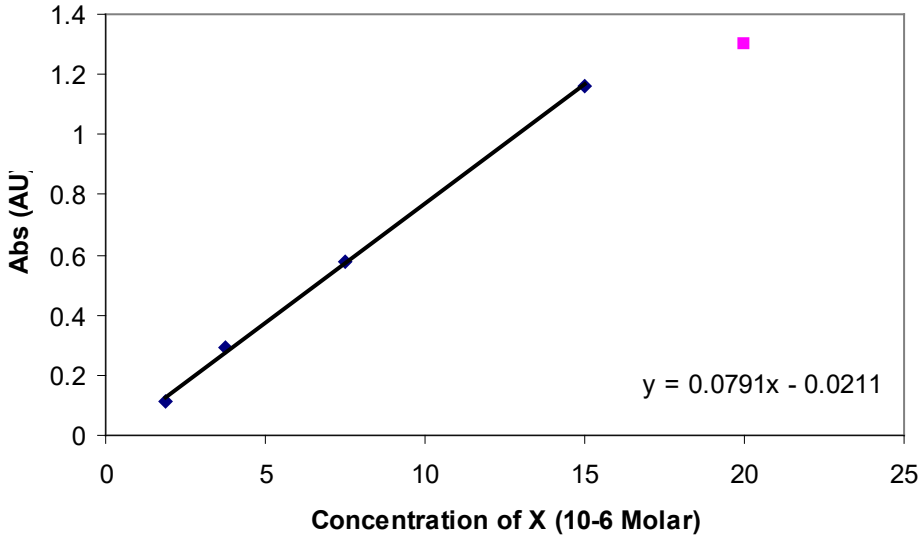
Both expressions indicate that the absorbance increases *linearly* as a function of concentration. Thus, if we choose the tall peak (λ_{max} = 415 nm) in Figure 2 and plot absorbance (**Abs**$_{@415\ nm}$) versus **[X]** (molar concentration of X), we should obtain a straight line. The data abstracted from the UV-vis spectra are listed in Table 1 and plotted in Figure 3. As can be seen in the plot, the data does appear linear with the exception of the absorbance measurement at the highest concentration ([X] = 20.0 x 10^{-6} M). This is not uncommon and simply reflects that at high concentrations Beer's Law is not always obeyed by all samples, it only holds true for dilute solutions. If we ignore this data point (because it clearly is not following Beer's law) and fit a line to the remaining data points,

TABLE 1

[X] (x10^{-6} M)	**Abs**$_{415\ nm}$
1.87	0.11
3.75	0.29
7.50	0.58
15.0	1.16
20.0	1.3

we can obtain the slope for the linear region which is equal to the molar extinction coefficient (**ε**) for compound X at 415 nm. In this case, the ε_{415nm} = 79,100 L/mol·cm. The molar extinction coefficient is an inherent property of the molecule (compound X in this case) and reflects how much light of wavelength 415 nm is absorbed by the molecule.

FIGURE 3: Beer's Law Plot of **Abs**$_{415\ nm}$ vs. **[X]**



Thus, anyone who prepares compound X anywhere in the world should obtain the same value (ε_{415nm} = 79,100 L/mol·cm) for compound X at this wavelength. Of course, we could choose any wavelength. Inspect Figure 2 and you will see that at any wavelength the absorbance decreases linearly with concentration. By convention, we measure and report **ε** values at peaks in the absorption spectrum. For your pre-lab, you will calculate **ε** for compound X at the other peak (wavelength = 610 nm).

NOTE: The spectrometer has limits in which absorbance values are considered reliable. Absorbance's below 0.05 are too weak to be measured accurately. Absorbance's above 1.5 are too concentrated to be reliable. This reflects that the concentration of the colored species is too high and the solution is opaque (not transparent). Typically we simply dilute solutions with high absorbance readings to get the readings between 0.05 and 1.5 OD. OD stands for optical density and is unitless. As seen in Figures 1 and 2, absorbances are often reported with the units A.U. which stands for

arbitrary units. Because there is no unit to OD, we can arbitrarily choose any scale we want, thus the label A.U.

Three important applications of Beer's Law and Molar Extinction Coefficients!

1) The direct relationship between concentration and absorbance makes it possible to determine the **ε** by simple absorbance measurements of a solution of known concentration.
2) If the value of **ε** at λ_{max} for a compound is known, we can quickly determine the concentration of a solution of that compound by recording the absorbance at the λ_{max} and using the expression $A=\varepsilon bc$.
3) We can compare the ε value for a freshly prepared compound to the published (literature) value. If the two match, we can conclude the sample is pure. However, if the freshly prepared sample has a smaller ε we can conclude that the new sample is not pure and can estimate its purity from the expression:

$$\% \text{ Purity} = \frac{\varepsilon \text{ sample}}{\varepsilon \text{ literature}} (100\%) \qquad (3)$$

In this experiment you will use an UV-Visible Spectrophotometer to record the absorption spectrum of your colored $[Fe(phen)_3]Cl_2$ compound in dilute aqueous solution. You will prepare a series of solutions of this complex in water and record the absorption spectrum between 400 and 700 nm. From the spectra, you will determine the λ_{max} and record the absorbance values at this wavelength for the solutions at various concentrations. You will construct a table of concentration of $[Fe(phen)_3]Cl_2$ and Absorbance (like Table 1) and plot the data in Excel to give a Beer's Law plot (Figure 3). By examining the plot, you will determine the linear region of the plot and draw a best fit line to that region. From the slope of this line, you will determine ε for the $[Fe(phen)_3]Cl_2$ complex and compare it to the reported value. The literature value for $[Fe(phen)_3]Cl_2$ in water is $\varepsilon = 9700$ L/mol·cm at the highest peak in the 400 to 700 nm range.

Finally, you will be given a solution of $[Fe(phen)_3]Cl_2$ (aq) and asked to determine its concentration using the spectrophotometer and Beer's Law.

Hazardous waste disposal: All the solutions should be combined and disposed of in the Heavy Metals Waste container.