Color and the Absorbance of Light by Molecules

Submit your answers to the questions found at the end of the prelab handout to the instructor at the beginning of the laboratory. You will not be allowed to begin work without having done so. Please include printout from any calculations done using EXCEL or another program.

Objectives
After completing these exercises, you will understand both the wave and particle nature of light and how the absorbance of light by solutions results in the color that we perceive. You will learn how to use spectrophotometers to measure the absorbance of light by solutions as a function of wavelength. The spectrophotometers will be used to determine the concentration of an unknown solution.

Goals
- To understand both the wave and particle nature of light.
- To understand the relation between light absorbance and the perceived color of a solution.
- To learn how to use a diode-array spectrophotometer and the Spec-20 spectrophotometer.
- To understand the mathematics of concentration and dilution.
- To make a Beer’s Law plot.
- To determine the concentration of an unknown solution colorimetrically.

Lab Summary
The absorbance spectra of different color dyes will be collected. The spectra will be used to derive a relationship between the light absorbed by a solution and the color of the solution that we perceive. A spectrum will be taken of a colored metal ion stock standard solution to determine the wavelength of maximum absorbance. The stock solution will be diluted several times and the absorbance for each dilution will be measured at the wavelength of maximum absorbance. This data will be used to make a Beer’s Law plot that can be used to determine the absorbance of a solution of unknown concentration.

Prelab Background
Light behaves both as a particle and a wave. At first this may seem funny but get used to it please. Many of the tiny things on the atomic scale, such as electrons and protons, which chemists and chemistry students have to think about, have this peculiar duality.

Wave Nature of Light
The wave nature of light is seen when light rays are diffracted through two closely spaced slits to form a pattern of bands of constructive and destructive interference. The light has the wave-like properties of wavelength and frequency. Wavelength is the distance between any two crests or troughs on a wave. The arrows on the waveform below each indicate one wavelength of the wave. The wavelength of visible light ranges from 400x10^{-9} m or 400 nm (nm stands for nanometer, a unit of length, 1 \text{ nm} = 1\times10^{-9} \text{ m}) for blue light to 750x10^{-9} m or 750 nm for red light.
Light waves have a **frequency**. The frequency is how many wavelengths or oscillations occur per second. Frequency has units of per second or sec\(^{-1}\). Wavelength and frequency are related to each other by the equation \( c = \lambda \nu \), where \( \lambda \) is the Greek letter lambda, the symbol for wavelength, \( \nu \) is the Greek letter nu, the symbol for frequency and \( c \) is the symbol for the speed of light. The speed of light is a constant with a value of \( c = 3.0 \times 10^8 \text{ m/sec} \) for all wavelengths of light. Since the product of wavelength and frequency is a constant, light that has a longer wavelength will have a lower frequency in order to move at the same speed as light that has a shorter wavelength and therefore must have a higher frequency. This is shown in the two example calculations below.

**Example:** What is the frequency of red light with a wavelength (\( \lambda \)) equal to 750 nm?

Rearrange \( c = \lambda \nu \), to solve for frequency by dividing both sides of the equation by wavelength (\( \lambda \)).

\[
\nu = \frac{c}{\lambda} = \frac{3.0 \times 10^8 \text{ m/sec}}{(750 \text{ nm})(1 \times 10^{-9} \text{ m/1 nm})} = 4.0 \times 10^{14} \text{ sec}^{-1}
\]

Note how the units of the equation obey the rules of mathematics and show us that we have the correct answer with units of frequency (sec\(^{-1}\) or 1/sec).

\[
\text{nm}*\text{m/nm} = \text{m as nm on top and bottom cancel out}
\]

\[
\text{m/sec/ m} = 1/\text{sec or sec}^{-1} \text{ the units of frequency as m top and bottom cancel.}
\]

**Example:** What is the frequency of ultraviolet light with a wavelength (\( \lambda \)) equal to 375 nm?

Rearrange \( c = \lambda \nu \), to solve for frequency by dividing both sides of the equation by wavelength (\( \lambda \)).

\[
\nu = \frac{c}{\lambda} = \frac{3.0 \times 10^8 \text{ m/sec}}{(375 \text{ nm})(1 \times 10^{-9} \text{ m/1 nm})} = 8.0 \times 10^{14} \text{ sec}^{-1}
\]

Notice how in the two example calculations that when the wavelength decreases by a factor of two on going from 750 nm to 375 nm that the frequency increases by a factor of two, going from \( 4.0 \times 10^{14} \text{ sec}^{-1} \), for the light with the longer wavelength (750 nm), to \( 8.0 \times 10^{14} \text{ sec}^{-1} \) for the light with the shorter wavelength (375 nm).

**Particle Nature of Light**

Light also has particle like properties. The most important is that the energy of light is **quantized**. This means that the energy comes in specific quantities, much like milk comes in quarts, half-gallons and gallons at the grocery store. The quantities of energy that light comes in are called photons. The energy of a photon depends on the frequency of the light. The energy of
the photon is given by the equation \( E = h \nu \), where \( h \) is called Planck's constant and has a value of \( h = 6.626 \times 10^{-34} \) Joule - sec and \( \nu \) is frequency with units of sec\(^{-1}\).

Example: What is the energy of a photon of light with a wavelength of 800 nm in Joules (J)?

We need to first calculate the frequency of the light.

\[ \nu = \frac{c}{\lambda} = \frac{3.0 \times 10^8 \text{ m/sec}}{(800 \text{ nm})(1 \times 10^{-9} \text{ m/1 nm})} = 3.75 \times 10^{14} \text{ sec}^{-1} \]

Now we can calculate the energy

\[ E = h \nu = 6.626 \times 10^{-34} \text{ Joule - sec} \times 3.75 \times 10^{14} \text{ sec}^{-1} = 2.48 \times 10^{-19} \text{ Joules} \]

Note how the units of sec cancel out to leave Joules, which are units of energy.

Light of a specific wavelength is only found in quanta or chunks of energy that are multiples of the photon energy for that wavelength. A photon of light however is a very small quantity of energy and this is why things appear continuous (available in any quantity of energy) and not quantized. The step size is so tiny that we do not perceive the steps and we need special instruments and experiments to determine that red light of 800 nm is available only in energies that are whole number (1, 2, 3, 4...) multiples of 2.48x10\(^{19}\) Joules. It is sometimes convenient to talk about the energy of a large number of photons, Avogadro's number of photons or 6.02x10\(^{23}\) photons or a mole of photons. If we have a mole of 800 nm photons they have an energy of 149,582 J/mole or 1.5x10\(^{5}\) J/mole or 150 kJ/mole as shown in the example below.

Example: What is the energy of a photon of light with a wavelength of 800 nm in Joules/mole?

Multiply the energy of a photon by 6.02x10\(^{23}\) photons/mole

\[ 2.48 \times 10^{19} \text{ Joules/photon} \times 6.02 \times 10^{23} \text{ photon/mole} = 149,582 \text{ J/mole} \]

1 kJ (kiloJoule) = 1000 J

\[ 149,582 \text{ J/mole} \times (1 \text{ kJ/1000 J}) = 150 \text{ kJ/mole} \]

**Light and Color**

In the previous experiment light of specific wavelengths or color was observed being emitted by atoms undergoing a transition from an excited energy state to a lower energy state. The lower energy state is often the electronic ground state. The **electronic ground state** of an atom is when the electrons are arranged in the atomic orbitals in the lowest energy possible. An **excited electronic state** of an atom is an arrangement of electrons in the atomic orbitals at energies greater than the lowest possible energy. The energy released by the atom in the form of a photon was detected by your eye and interpreted by your brain as a color.

In this experiment we will be investigating the absorbance, and not the emission of photons, by atoms and molecules. Atoms and molecules only absorb photons of light if the energy of the photon is equal to the energy difference between the ground state and an excited state. The absorbed photon will supply the energy for the transition of the atom or molecule from the ground state to the higher energy excited state. Transitions are also possible from excited states to even higher energy excited states, but the experiment necessary to detect this requires high powered lasers to supply the needed photons.

When a beam of white light collides with an object, the object absorbs some of the light and the rest of the light reflects off the object and can be detected by our eyes. Visible light is part of the electromagnetic spectrum. Our eyes can detect only light with wavelengths between 750 and 400 nm. The different wavelengths of light between 750 and 400 nm that are reflected and absorbed by objects are responsible for the colors that we see.
The Electromagnetic Spectrum

The spectrophotometer has three main components: a light source, a dispersive element and a light detector. The light source shines through a slit that produces a narrow beam. The narrow beam of light shines onto the dispersive element. In older machines the dispersive element is a prism, on newer machines the dispersive element is a diffraction grating. The dispersive element causes the narrow beam of light to be spread out into a spectrum or rainbow of colors. The different wavelengths are dispersed in space with each color taking a different path.
In the Spectronic-20 only one color of light, **monochromatic** light, makes it through the second slit to shine through the sample and hit the detector. The dispersive element is mounted on a pivot and as it rotates different colors will pass through the second slit. The detector in the Spectronic-20 is a device called a photomultiplier tube (PMT) that gives out a current proportional to the number of photons striking it. This current is measured and divided by the background to give us a measurement called the **absorbance**. A higher absorbance means less light is hitting the detector and more light is being absorbed by the sample.

In the HP8453 the detector is a diode-array. There is no second slit and the dispersed light is allowed to illuminate a set of over a thousand diode detectors fabricated on a silicon chip. The large number of detectors allows the instrument to measure the absorbance at many different wavelengths of light simultaneously.

The diode array spectrophotometer will show spectra that contain broad absorbance bands in contrast to the narrow emission lines observed in last week’s experiment. This is due to the difference between being in a condensed phase such as a liquid solution and being in the gas phase. In a condensed phase there are lots of collisions between molecules, the solvent molecules keep crashing into the solute molecules. A collision will slightly shift the energy of an electronic state as the collision disturbs the distribution of the electrons in the molecule. All of the collisions produce a distribution of molecules with slightly different energies since some molecules are undergoing more collisions than other molecules at the instant that we collect the spectrum. All of the different states of the molecules result a broad band for a transition between electronic states. In the gas phase the molecules are widely separated and have very little interaction. There are very few collisions. As a result all of the molecules are pretty much undisturbed and at the same energy. The transition between states results in a narrow emission band.

**Solutions and Dilution**

A solution is made of two components: the solute, what is dissolved and the solvent, the medium in which the solute is dissolved. In this experiment the solutes are the various dye molecules and metal ions while the solvent is deionized water in both cases. A very important parameter for a solution is its concentration. Concentration is defined as the number of particles of solute in a volume of solution. Concentration has units of **Molar** or **moles/liter of solution**. Molar is abbreviated using “M”.

\[
\text{Concentration} = \frac{\text{Moles}}{\text{Volume (l)}}
\]
Example: What is the concentration if 400 moles of solute are dissolved to make 375 liters of solution?
Concentration = 400 moles / 375 liter = 1.07 mole/liter = 1.07 Molar = 1.07 M

Example: What is the concentration if 4.00 moles of solute are dissolved to make 375 milliliters of solution?
Convert milliliters to liters: 375 ml * (1 l/1000 ml) = 0.375 l as ml cancel top and bottom.
Concentration = 4.00 moles / 0.375 liter = 10.67 mole/liter = 10.67 Molar = 10.67 M

When additional solvent is added to the solution in a dilution the solution’s volume increases as the solution’s concentration decreases. Since no solute is added to the solution during a dilution the number of moles of solute remains constant. The equation that expresses this mathematically is:

\[ M_{\text{old}} V_{\text{old}} = M_{\text{new}} V_{\text{new}} \]

Where M and V are solution molarity and volume and the old subscript indicates the solution before the dilution and new subscript indicates the solution after dilution.

A dilution can also be indicated by a volume ratio such as 1:10. This ratio indicates that 1 part of the old solution will be used to make a new solution with a volume of 10 parts. In order to make the dilution 9 parts or 9 times the volume of the original solution worth of solvent need to be added to the 1 part of the old solution.

Example: A 0.1 M NaCl solution with a volume of 150 ml is diluted to a volume of 450 ml.
What is the concentration of the new solution?
\[ M_{\text{old}} = 0.1 M, V_{\text{old}} = 150 \text{ ml}, V_{\text{new}} = 450 \text{ ml} \]
Rearrange \( M_{\text{old}} V_{\text{old}} = M_{\text{new}} V_{\text{new}} \) to solve for \( M_{\text{new}} \).
\[ M_{\text{new}} = \frac{M_{\text{old}} V_{\text{old}}}{V_{\text{new}}} = \frac{0.1 \text{ M} \times 150 \text{ ml}}{450 \text{ ml}} = 0.033 \text{ M} \]

Example: A 0.1 M NaCl solution with a volume of 150 ml has to be diluted to a concentration of 0.025 M. What volume of solvent is needed? What is the dilution ratio?
\[ M_{\text{old}} = 0.1 \text{ M}, V_{\text{old}} = 150 \text{ ml}, M_{\text{new}} = 0.025 \text{ M} \]
Rearrange \( M_{\text{old}} V_{\text{old}} = M_{\text{new}} V_{\text{new}} \) to solve for \( V_{\text{new}} \).
\[ V_{\text{new}} = \frac{M_{\text{old}} V_{\text{old}}}{M_{\text{new}}} = \frac{0.1 \text{ M} \times 150 \text{ ml}}{0.025 \text{ M}} = 600 \text{ ml} \]
Since the volume of the new solution is 600 ml the volume of solvent needed is 600 – 150 = 450 ml since the new solution is made from the old solution and the added solvent.
The dilution ratio is \( V_{\text{old}}:V_{\text{new}} = 150:600 = 1:4 \).

**Beer’s Law**

Beer’s Law states that the absorbance \( (A) \) of a solution is proportional to or a linear function of the concentration \( (c) \) of the solute in solution: \( A \propto c \). The higher the concentration of the solute, the greater the probability of a photon of light being absorbed as it passes through the solution and the higher the absorbance. The proportionality sign can be converted into an equal sign by inserting the path length of the light through the solution \( (l) \) and the molar extinction coefficient \( (\varepsilon) \) of the solute into the proportionality to yield: \( A = \varepsilon cl \).

The molar extinction coefficient is a function of wavelength; at every wavelength a molecule of solute has a different molar extinction coefficient. The larger the molar extinction coefficient the more effective the solute is at absorbing light. If the molar extinction coefficient is known it is then possible to calculate the concentration of a chromophore in solution from absorbance data.
The molar extinction coefficient is equal to the slope of the line used to fit your data (a trendline in EXCEL) on a graph of absorbance at a given wavelength vs. solute concentration. This type of graph is known as a Beer’s Law Plot. Note that in the plot below the blank vs. blank absorbance point is included in the data, as it is expected that the absorbance will be equal to zero with no solute present. The extinction coefficient for this solute is equal to 69.1 M$^{-1}$cm$^{-1}$ at 530 nm and the quality of the data is good as the goodness of fit parameter ($R^2$) is very close to 1.00. A value of 1.00 indicates that all of the data points lie on the fitted line and a value of 0.00 indicates that the data displays only random scatter.

![Beer's Law Plot: Data Collected at $\lambda = 530$ nm](image)

Example: Using the above graph what is the concentration of a solution that has an absorbance of 0.27 absorbance units?
The equation will give us the most precise answer. Absorbance is on the y-axis so the equation becomes: $0.27 = 69.101x - 0.0012$.
Solving for $x$ gives a concentration of $3.9\times10^{-3}$ M.

Example: Using the above graph what is the absorbance of a solution with a concentration of 0.0057 M?
The equation will give us the most precise answer. Concentration is on the x-axis so the equation becomes: $y = 69.101\times0.0057 - 0.0012$.
Solving for $y$ gives an absorbance of 0.393.
Prelab Questions
1) What is the frequency of a photon that has a wavelength of 650 nm?
   That has a wavelength of 325 nm?
2) What is the energy of a photon that has a wavelength of 650 nm in J and J/mole?
   That has a wavelength of 325 nm in J and J/mole?
3) What is the wavelength of a photon that has a frequency of $3.75 \times 10^{14}$ sec$^{-1}$?
   That has a frequency of $7.50 \times 10^{14}$ sec$^{-1}$?
4) What is the concentration if:
   a) 1 mole of solute is dissolved to make 2 liters of solution?
   b) 0.5 mole of solute is dissolved to make 2 liters of solution?
   c) 0.05 mole of solute is dissolved to make 20 milliliters of solution?
   d) $3 \times 10^{-3}$ mole of solute is dissolved to make 15 milliliters of solution?
   e) $3.01 \times 10^{-21}$ molecules of solute are dissolved to make 1 liter of solution?
5) A 0.05 M NaCl solution with a volume of 125 ml is diluted to a volume of 750 ml. What is the concentration of the solution?
6) A 0.05 M NaCl solution with a volume of 125 ml has to be diluted to a concentration of 7.1x10-3 M. What volume of solvent is needed? What is the dilution ratio?