Online Activity 2
Spectrophotometry

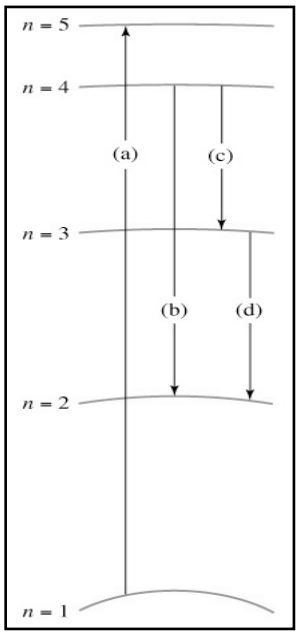
Introduction

The flash of fireworks, the warm glow of a campfire, the bright color of a neon sign – these phenomena are a welcome part of our everyday lives, yet most of us are unfamiliar with the way the light is produced. Where does the color come from? How is it related to the energy of the system? Why is the color of the flame from the campfire different from the sparkling of the fireworks or the blue flame of a gas stove? Why is the fire hot and yellow, yet the neon tube looks blue and feels cool? What causes the light that you see and why does it have a particular color? These questions can be answered by studying the atomic absorption and emission properties of a system and the type of energy absorbed and then released when atomic emission has occurred.

Atomic Absorption and Emission

When atoms are exposed to energy, such as visible or ultraviolet light, their **valence electrons** can absorb specific amounts of that energy (**atomic absorption**), which allows them to move from their lowest stable energy level, called the **ground state**, to a higher energy level, referred to as the **excited state.** The absorbed energy can then be released through a process called **emission**. Valence electrons are the electrons in the outer shell of an atom. They are responsible for any reactivity in the atom and for the color changes seen because of absorption and emission.

The energy needed to raise the valence electrons in an atom to an excited state may come from many sources, such as light, an electric discharge, or heat. Energy is later emitted when the excited electrons fall back to lower shells and is often released in the form of light. The wavelength and color of the emitted light depends on the amount of energy originally absorbed by the atoms. Each excited electron emits a single wavelength of light, which, if the wavelength emitted is in the visible range, will correspond to a specific color. The wavelength of light emitted is characteristic of the change in energy level of the particular electron associated with the release of the added energy. Not all electrons in a sample will absorb or release energy in the same manner.



For example, in hydrogen gas, the ground state of a hydrogen atom has a single electron in the n = 1 shell. When energy is added, this electron can absorb a certain wavelength of light (absorption) and be excited into the n = 2 level. Higher amounts of energy of specific wavelengths can promote the electron into even higher energy levels. Since larger atoms have more electrons, they have many more transitions available to them, and electrons are often excited into the n = 3, 4, or 5 shells. The energy can then be released (emission) as the electron drops to one of the shells below. The amount of energy, and thus the color, emitted depends on the difference in energy between the higher energy level that the electron was excited into and the lower energy level that it returns to.

For both absorption and emission, each individual electron's change in energy is associated with a specific wavelength. When atoms in a gaseous sample of an element are excited, many of the wavelengths of emitted light are in the visible range, resulting in several different colors being observed in the gas. When observed through a prism, these colors are separated into a series of lines. The pattern of lines corresponding to the wavelengths of light emitted by an element for all possible electron transitions is called an **emission spectrum**. Since there are multiple atoms in a sample, and each atom or molecule usually has more than one electron, the color we see is a mixture of all the different **wavelengths** of light released by all the excited electrons in the sample of atoms. Each

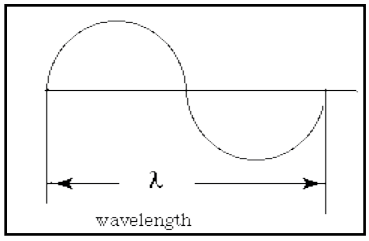
element has a different set of emission wavelengths, and thus colors, because they have different distances between their energy levels; therefore, each element will have a characteristic spectrum when its electrons are excited by the addition of energy. These characteristic emission spectra can be used for the identification of elements, and have aided in the study of distant stars and metal containing samples on earth.

The Relationship between Wavelength, Frequency and Energy

All light travels at the same speed, 3.00 x 10^8 meters/second, but the **frequency** of the light, or number of the light pulses per second, varies based on the wavelength (the distance from 1 identical point in a wave to the next). Therefore, frequency is related to wavelength through the formula:

$$c = \lambda\nu$$

c is the speed of light (3.00 x 10^8 m/s or m s^{-1})
Nu, **ν,** is the frequency in cycles per second (s^{-1})
Lambda**, λ,** is the wavelength in meters.



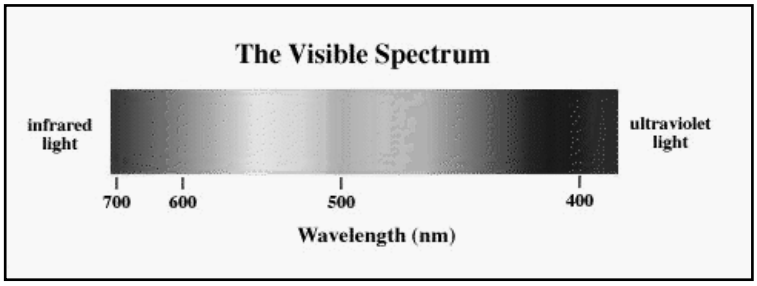
The frequency,ν, is equal to the number of cycles that pass an observer in 1 second. Each individual cycle contains a specific amount of energy carried by a single light particle called a **photon.** The energy of a photon is different for each color. We can calculate the energy of a particular photon using the relationship between the wavelength and the energy with a proportionality constant called Planck's constant, **h**, which equals 6.626 X 10^{-34} J s. The energy of the photon can then be calculated from the wavelength through the following relationship:

E = hc/λ where h = Planck's constant, (6.626 X 10^{-34} J s)

The Relationship between Color and Wavelength

All electromagnetic energy is associated with a characteristic wavelength. The wavelength is the distance between the beginning and end of a complete cycle of the light wave.

Visible light has wavelengths in the range of 390-760nm. The spectrum of all the colors and wavelengths of visible light is referred to as the **visible spectrum** and is the range of wavelengths associated with human vision.



The lower energy side of the visible spectrum is associated with red light. Red light has longer wavelengths and is lower in energy than blue light. The wavelength of red light corresponds to

the range of 760 to 600 nanometers or 0.00000076 – 0.0000006 meters). Blue light is associated with the shorter wavelengths in the range of ~400 nm or ~0.0000004 meters.

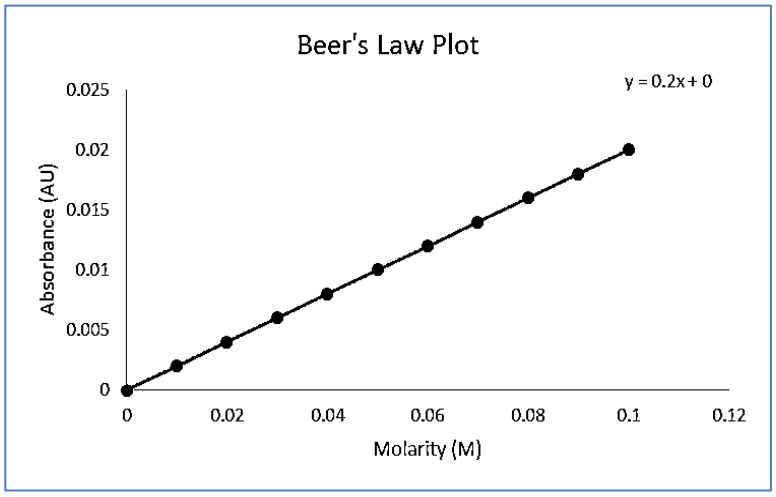
Atomic Absorption and Beer's Law

Each atom in a sample absorbs a fixed amount of energy based on how many electrons can be excited to a higher state. The more atoms you have, the more the light can be absorbed. This relationship between the amount of a substance and absorption is linear (at least at low concentrations), and thus it can give a researcher an indication of the number of atoms in a sample, which is directly related to the concentration in moles/L. In general, the darker the color, the more atoms are in the sample, which enables more light to be absorbed. The relationship between concentration and the absorbance of energy is called **Beer's Law**:

$$A = \varepsilon bc$$

Beer's Law is an equation that links the absorbance of energy by a solution to the concentration of that solution. The variables are as follows:

A is the absorbance of a solution being measured
ε is a proportionality constant called the **molar absorptivity**
b is the path length of the cuvette, usually 1cm
(Long path length: more atoms are in the cuvette, so the absorbance increases)
c is the concentration of the solution in Molarity



*Note that the Beer's Law relationship is only linear over a range of relatively low concentrations. As a result, spectrophotometric analyses require dilute solutions.

Spectrophotometric Analysis

Spectrophotometry is a method used to determine how much light is absorbed by a sample. A **spectrometer** measures the absorbance of light by a sample and gives a numerical reading that correlates to the sample's concentration. A spectrometer works by shining a known amount of light through a sample and measuring how much light comes out through the other side (the amount of light that is "transmitted"). The "**absorbance**" is a measurement of the amount of light that does not come out, but rather is absorbed by the atoms in the sample. This is analogous to shining a light through a glass of water versus a glass of grape juice. The grape juice, being darker, will absorb more light, thus the amount of light coming out the other side of the glass is less than that seen through a glass of water. Grape juice has a higher absorbance than water.

A spectrometer assigns numerical values that correspond to the amount of light and the specific wavelength of light that is absorbed by a solution. These absorbance values can be correlated to

the concentration of the solution. A colorless solution will allow visible light to go through uninhibited and therefore the value of the absorbance will be very small; ideally it is zero. As the concentration of a colored solution increases, it gets darker and absorbs more visible light. The absorbance values will increase at a linear rate as the concentration of the solution increases. A **calibration curve** is used to determine exactly how much light is absorbed per molar unit (AU/M). A series of **standards** (solutions of known concentration) are prepared and inserted into the spectrometer to determine the absorbance of each. A plot of absorbance versus concentration of these standards will result in a graph similar to that shown on the previous page. This method is a very effective means of relating absorbance to concentration and gives a linear equation that can be used to find the concentration of an unknown solution by measuring its absorbance. (Know Y, solve for X)

In This Activity

You will be using a Phet simulation, developed by the University of Colorado at Boulder, to obtain the data for this activity. The procedure section will give you a concentration for your nickel stock solution. You will need to use the dilution calculation, which was described in the first online activity, to calculate concentrations for a series of diluted solutions, which will be your standard solutions. The simulation will allow you to enter the concentration of each nickel standard, and will give you an absorbance value for each solution. You will then graph these absorbance values vs. concentration to graph a Beer's Law plot as described in the introduction. You will then use the Beer's Law plot to determine the concentration of nickel in your unknown solution.

Online Activity 2: Procedures and Data Sheet

(Submit as part of your activity report)

Name: _____ Date: _____ Section: _____

Record all measurements with the correct number of significant figures and units.

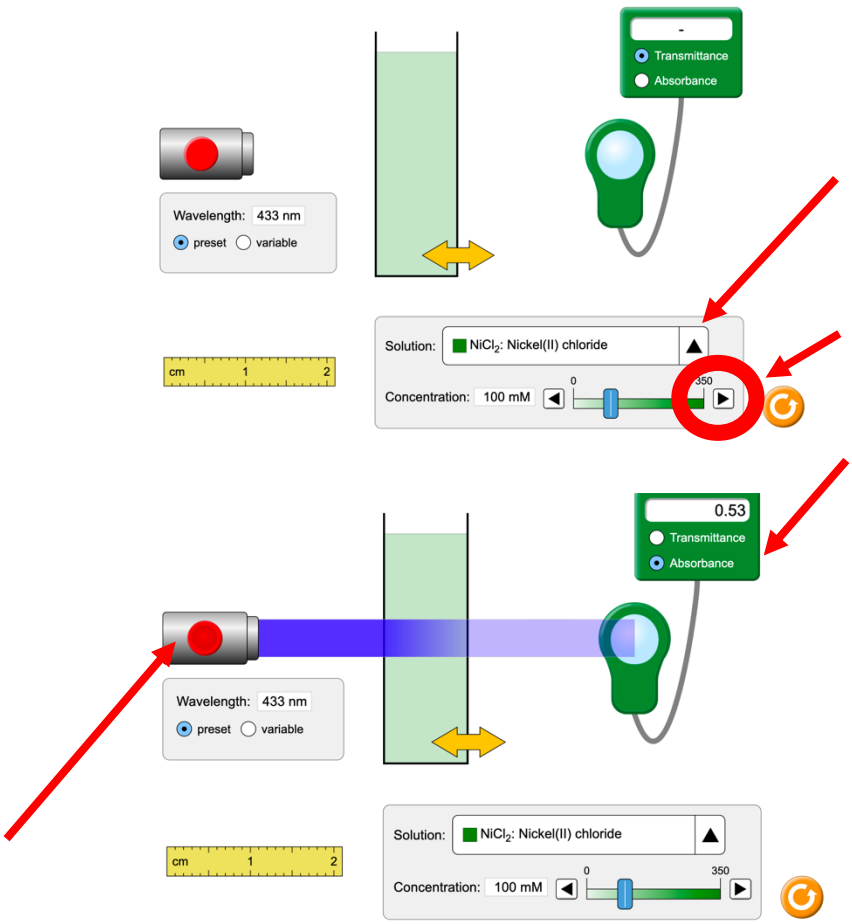
Part 1. Calculating the concentration of your nickel standards

You are starting with a stock solution containing 90.00 mg/mL Nickel(II) chloride (NCl_2; molar mass = 129.599g/mol).

1. Convert the mg/mL concentration into molarity (moles/L). Record this work in the calculations section.
2. To make your standard solutions, dilute the following volumes of stock solution to a total volume of 25.00mL. Convert the concentrations to millimolar (mM), which is what the simulation uses. Fill in the table with the concentrations, and show your work in the space below.

Standard	Volume $NiCl_2$ Stock solution	Total volume	Concentration of Standard (M)	Concentration of Standard (mM)
Blank	0.00mL	25.00mL		
STD1	1.00mL	25.00mL		
STD2	2.00mL	25.00mL		
STD3	3.00mL	25.00mL		
STD4	4.00mL	25.00mL		
STD5	5.00mL	25.00mL		

Once you have your standard concentrations, go to
https://phet.colorado.edu/sims/html/beers-law-lab/latest/beers-law-lab_en.html



Select Beer's Law. You should see a simulation that looks like the image at left.

The solution will initially be set to drink mix. Use the black triangle at the right to select $NiCl_2$ as your solution. Leave the wavelength on preset, and it will automatically change to 433 nm.

Use the arrows or the blue bar to change the concentration to the concentration that you calculated for your first standard. Change the setting at the top right to absorbance rather than transmittance, and click on the red button on the left. The absorbance value will appear in the green box. Record the absorbance value below. Repeat for the remaining standards.

Once you have absorbance readings for your standards, make a calibration curve as described in your lab manual. Use the equation of the line from that curve, **and an absorbance reading of 0.56 for your "test solution"** to calculate the concentration of nickel in your test solution.

Record the absorbance values for your standard solutions:

Absorbance of blank _____

Absorbance of STD1 _____

Absorbance of STD2 _____

Absorbance of STD3 _____

Absorbance of STD4 _____

Absorbance of STD5 _____

Online Activity 2: Data Rubric (20pts)

Points

Data are neat and legible	5pts	_____pts
Significant figures (>80% correct)	3pts	_____pts
Units (>80% correct)	2pts	_____pts
All data are present and make sense	10pts	_____pts

Deductions (sliding scale based on TA discretion)

Lab area left unclean	-20pts	_____pts
Improper waste disposal	-20pts	_____pts
Disruptive behavior	-20pts	_____pts
Lab coat or safety glasses removed while in lab	-20pts	_____pts
Data sheet is missing TA signature	-20pts	_____pts
Other: _____		_____pts

Comments: _____

Grade for Data Sheet **_____pts**

Online Activity 2: Results Table
(Submit as part of your activity report)

Name: _____ Date: _____ Section: _____

Record all results with the correct number of significant figures and units.

Table 1: Colorimetry Information

Wavelength Used:	
Frequency of wavelength used:	
Energy associated with Wavelength used:	

Table 2: Vitamin Standards

	Concentration of Iron	Absorbance
Blank		
STD1		
STD2		
STD3		
STD4		
STD5		
Equation of line from graph		

Table 3: Mass Calculations for the Vitamin Tablet

Absorbance value for the test solution (given in procedure!)	
Molarity of $NiCl_2$ in test solution (from graph)	
Molarity of $NiCl_2$ in original solution (before dilution)	
Moles of $NiCl_2$ in tablet	
Experimental mass (in g) of $NiCl_2$ in tablet	
Percent error in mass of $NiCl_2$	

You must also include a copy of your graph

The graph must have a title
Both axes must be correctly labeled
The equation for the line must be on the graph
The value of the correlation coefficient, R^2, must be on the graph

Online Activity 2: Results Table Rubric (20pts)

Points

Tables are neat and legible	5pts	_____pts
Significant figures (>80% correct)	3pts	_____pts
Units (>80% correct)	2pts	_____pts
All results are present and make sense	10pts	_____pts

Deductions (sliding based on TA discretion)

Results to not match data	-20pts	_____pts
Plagiarism!!! Results are identical to another student	**-100pts**	_____**pts**
Other: _____		_____pts

Comments: _____

Grade for Results Table _____**pts**

Online Activity 2: Calculations

(Submit as part of your activity report.)

Background calculations:
You used a wavelength of 433nm for your analysis. Use the speed of light calculate the frequency of this wavelength. Be careful with your units!

Frequency: _____

Use Plank's constant to calculate the energy associated with a wavelength of 433nm. Be careful with your units!

Energy: _____

Concentration of original stock iron solution in molarity
original stock solution of Nickel(I) chloride ($NiCl_2$, 129.599g/mol) is 90.00 mg/ml. Use dimensional analysis to convert the units to moles/L.

Molarity of stock $NiCl_2$ solution: _____

Concentration of standards
The work for these calculations should be shown in the procedure section.

Calibration curve
Construct a calibration curve using the scatter plot option in a spreadsheet program. Plot the concentration of the five $NiCl_2$ standards and the blank on the x axis (M) and the absorbance value of each solution on the y axis (abs). If you are using the version of excel provided by URI, make sure that you download it onto your computer rather than using the online version (the online version does not provide all the necessary functions).

Equation of line in calibration curve: _____

Molarity of $NiCl_2$ in the analyzed vitamin test solution
Use the value provided in the procedure section for your test solution as the y value in the equation of the line from your scatter plot. Solve for x which will be the concentration of the test solution in molarity.

Molarity of test solution: _____

Molarity of vitamin test solution before dilution

Assume that your test solution came from a vitamin, and that 10.00mL of the original vitamin solution was diluted to 50.00mL to produce the solution that was analyzed. Calculate the concentration of $NiCl_2$ in the original vitamin solution.

Molarity of $NiCl_2$ in original vitamin solution: _____

Moles of $NiCl_2$ in tablet

When the vitamin was processed, 100.00mL of solvent was used. You know the concentration of the solution from the previous step. Given that concentration and the total volume of 100.00mL, calculate the number of moles of $NiCl_2$ in the vitamin tablet.

Moles of $NiCl_2$ in tablet: _____

Experimental mass of $NiCl_2$ in tablet in mg

Use the moles of $NiCl_2$ to determine the mass of $NiCl_2$ in the vitamin tablet

Mass of $NiCl_2$ in tablet (g): _____

Percent error in mass of $NiCl_2$

Suppose the tablet was supposed to contain 6.500g $NiCl_2$. Calculate the % error in the mass.

% Error in mass of $NiCl_2$: _____