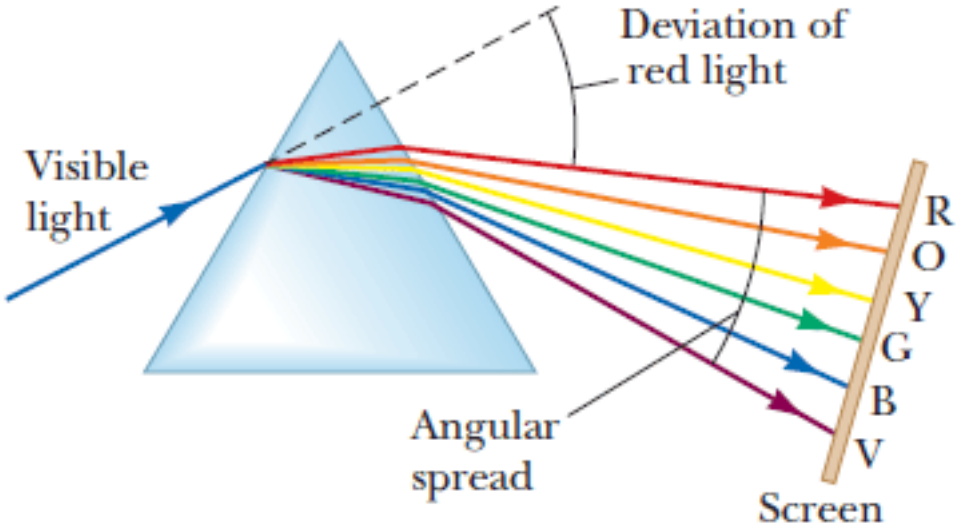
Spectroscopy of Food Dyes

Introduction:

The electromagnetic spectrum ranges from radio waves with wavelengths the size of buildings down to gamma rays, the size of atomic nuclei. White light forms a small part of this spectrum and is composed of a range of different wavelengths which can be dispersed using a prism into its component colors. The color an object, or a solution, appears will depend on which light is transmitted or reflected in the visible spectrum and which light is absorbed. Using a UV-visible spectrometer and a range of food dyes you will test how the absorbance wavelength value relates to the color of the solution.



UV – Visible Spectrometer:

UV-visible spectrometers can be used to measure the absorbance of ultra violet or visible light by a sample. The spectrum produced is a plot of absorbance versus wavelength (nm) in the UV and visible section of the electromagnetic spectrum. Instruments can be used to measure at a single wavelength or perform a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm. The technique can be used both quantitatively and qualitatively.

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.

Suppose you look at two solutions of the same substance, one a deeper color than the other. Your common sense tells you that the darker colored one is the more concentrated. In other words, as the color of the solution deepens, you infer that its concentration also increases. This is an underlying principle of spectrophotometry: the intensity of color is a measure of the amount of a material in solution.

A second principle of spectrophotometry is that every substance absorbs or transmits certain wavelengths of radiant energy but not other wavelengths. For example, chlorophyll always absorbs red and violet light, while it transmits yellow, green, and blue wavelengths. The transmitted and reflected wavelengths appear green—the color your eye "sees." The light energy absorbed or transmitted must match exactly the energy required to cause an electronic transition (a movement of an electron from one quantum level to another) in the substance under consideration. Only certain wavelength photons satisfy this energy condition. Thus, the absorption or transmission of specific wavelengths is characteristic for a substance, and a spectral analysis serves as a "fingerprint" of the compound.

In recent years spectrophotometric methods have become the most frequently used and important methods of quantitative analysis. They are applicable to many industrial and clinical problems involving the quantitative determination of compounds that are colored or that react to form a colored product.

LIGHT AND THE PERCEPTION OF COLOR

Light is a form of electromagnetic radiation. When it falls on a substance, three things can happen:

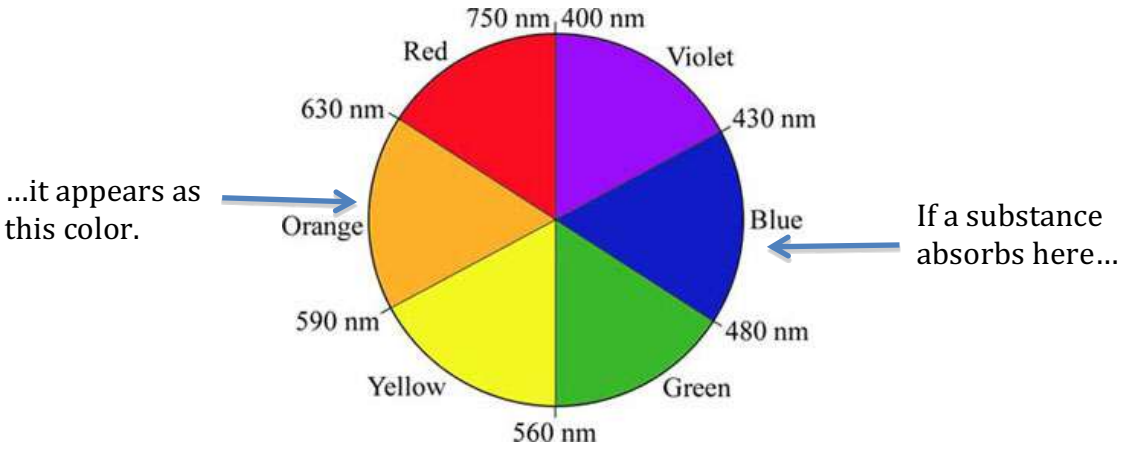
- the light can be reflected by the substance
- it can be absorbed by the substance
- certain wavelengths can be absorbed and the remainder transmitted or reflected

Since reflection of light is of minimal interest in spectrophotometry, we will ignore it and turn to the absorbance and transmittance of light.

The color we see in a sample of solution is due to the selective absorption of certain wavelengths of visible light and transmittance of the remaining wavelengths. If a sample absorbs all wavelengths in the visible region of the spectrum, it will appear black; if it absorbs none of them, it will appear white or colorless. We see the various colors when particular wavelengths of radiant energy strike our eyes. For example, the wavelength we perceive as green is 0.0000195 inches or, expressed more scientifically, 495 nanometers.

Suppose we shine a beam of white light at a substance that absorbs blue light. Since the blue component of the white light gets absorbed by the substance, the light that is transmitted is mostly yellow, the complementary color of blue. This yellow light reaches our eyes, and we "see" the substance as a yellow colored substance. The table below gives pairs of complementary colors and the corresponding wavelength ranges.

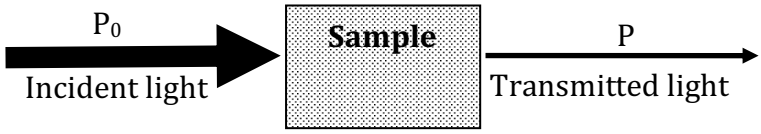
Wavelength (nm)	Color Absorbed	Color Observed
400	Violet	Yellow-green
435	Blue	Yellow
495	Green	Purple
560	Yellow	Blue
650	Orange	Greenish blue
800	Red	Bluish green



You should remember, of course, that the visible range is only a very small part of the electromagnetic spectrum. Ultraviolet and infrared spectrophotometric methods are suitable for many colorless substances that absorb strongly in the UV or IR spectral regions.

Transmittance, Absorbance, and the Beer-Lambert Law

We define transmittance as the ratio of the amount of light transmitted to the amount of light that initially fell on the surface.



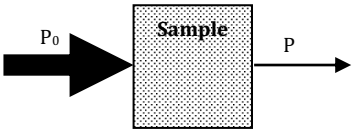
$$\text{Transmittance} = \frac{P}{P_0} = \frac{\text{intensity of transmitted light}}{\text{intensity of incident light}}$$

Absorbance is defined as the negative logarithm of the transmittance, and you will note that absorbance and transmittance bear an inverse relationship.

$$\text{Absorbance} = -\log T = -\log\frac{P}{P_0} = 2 - \log(\%T)$$

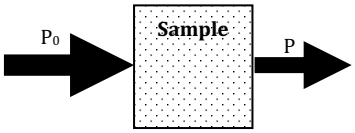
Going back to our example of chlorophyll, if you have two colored solutions, you may deduce that the darker colored green solution appears darker because it absorbs more of the light falling on it. Because the darker solution is also the more concentrated one, you can also say that the more concentrated one absorbs more of the light. That is, *the absorbance increases as concentration increases*.

In very basic terms, Absorbance and Transmittance are opposites or inverses of the other. If the Transmittance (T) is high, then the Absorbance (A) is low and vice versa.



Most of the incoming light is absorbed:

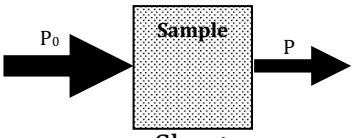
- Low Transmittance
- High Absorbance



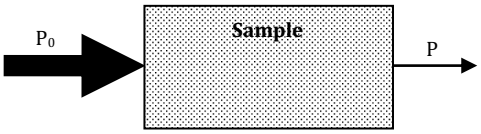
Most of the incoming light passes through the sample:

- Hight Transmittance
- Low Absorbance

Next, suppose that there are two test tubes, both containing the same solution at the same concentration. The only difference is that one of the test tubes has a longer path than the other. We shine light of the same intensity (P_0) on both containers. In the first case the light has to travel through only a short distance, whereas in the second case it has to pass through a much longer length of the sample. We might deduce that in the second case more of the light will be absorbed or cut off, since the path length is longer. In other words, absorbance increases as path length increases

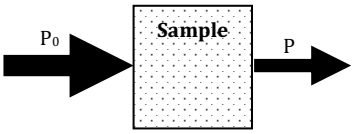


Short path

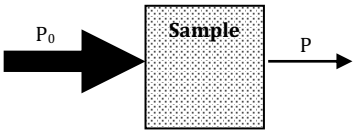


Long path

Finally, suppose that there are two test tubes, both containing the same solution and have the same distance to travel. The only difference is that one of the test tubes has a higher concentration than the other. We shine light of the same intensity (P_0) on both containers. In the first case the light has to travel through less particles (lower concentration), whereas in the second case it has to pass through much more particles (higher concentration) in the sample. We might deduce that in the second case more of the light will be absorbed or cut off, since more of the particles are absorbing the light. In other words, absorbance increases as the concentration increases.



Low concentration



High concentration

The two observations described above (those dealing with the relationship between absorbance and path length and absorbance and concentration) constitute the **BEER-LAMBERT LAW**.

Absorbance α path length (ℓ) · concentration

$$\boxed{A = \varepsilon \bullet \ell \bullet c}$$

where:

- A is a dimensionless number.
- ε the proportionality constant, is called the molar extinction coefficient or molar absorptivity. It is a constant for a given substance, provided the temperature and wavelength are constant. It has units of $\frac{L}{mol \cdot cm}$.
- ℓ have the units of length (cm)
- c have the units of concentration ($\frac{mol}{L}$ or M).

The quantitative measurement of light absorption as a function of wavelength can establish both the identity and the concentration of a substance in solution. The spectrophotometer is an instrument that separates electromagnetic radiation into its component wavelengths and selectively measures the intensity of radiation after passing through a sample. In this laboratory assignment you will use what is known as a "Spec 20" or Spectronic 20. Its operation will be demonstrated.

1. Plotting Absorption Spectra

Recall that the extinction coefficient for any given substance is a constant only so long as the wavelength of light is constant. You will see that the absorbance changes with wavelength.

The plot of a sample's absorbance of light at various wavelengths is called its absorption spectrum. (The abscissa or horizontal axis may be expressed in terms of wavelength and the ordinate or vertical axis in terms of absorbancy.) The plot below gives the absorption spectrum of potassium permanganate ($KMnO_4$), a purple colored solution, at two different concentrations. Curves 1 and 2 represent the absorption spectra measured under the same conditions except that curve 1 represents a more concentrated solution than curve 2. Note the similar shapes of the curves.

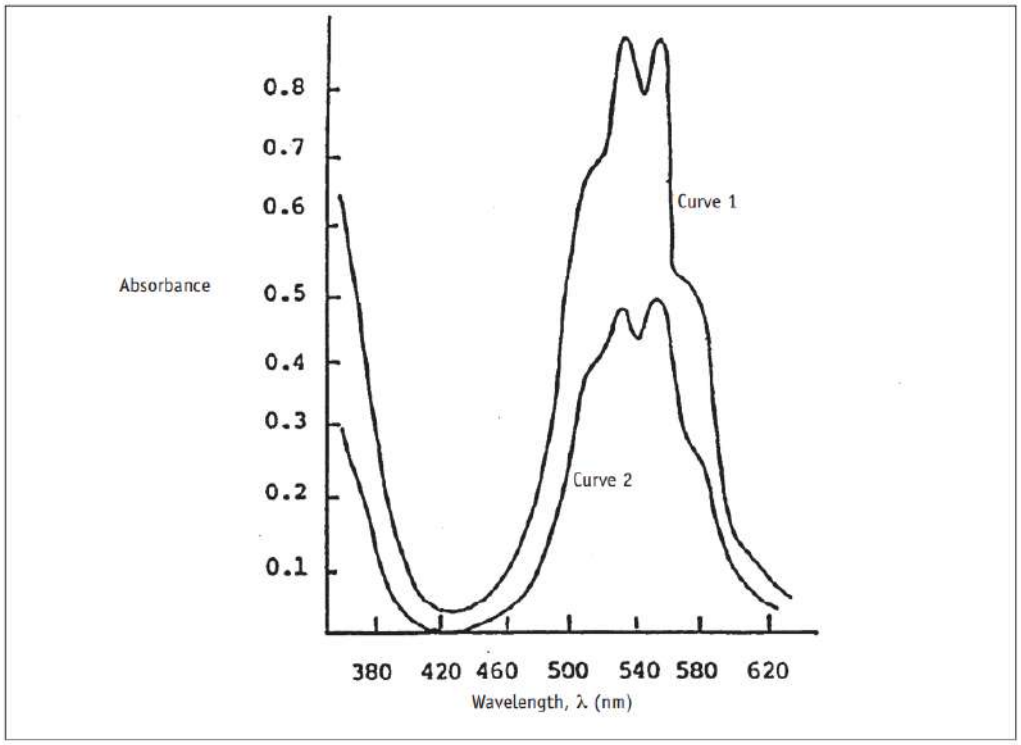


FIGURE The absorption spectrum of solutions of potassium permanganate ($KMnO_4$) at two different concentrations. The solution for curve 1 has a *higher* concentration than that for curve 2.

2. Choice of Wavelength

According to the Beer-Lambert Law absorbance is proportional to concentration at each wavelength. Theoretically we could choose any wavelength for quantitative estimations of concentration. However, the magnitude of the absorbancy is important, especially when you are trying to detect very small amounts of material. In the spectra above note that the distance between curves 1 and 2 is at a maximum at 525 nm, and at this wavelength the change in absorbance is greatest for a given change in concentration. That is, the measurement of concentration as a function of concentration is most sensitive at this wavelength. For this reason *we generally select the wavelength of maximum absorbance for a given sample and use it in our absorbance measurements.*

Suppose instead that we had chosen a wavelength of 500 nm for our measurement, this wavelength being on one of the steep portions of the curve. Examination of the curve shows that even a small fluctuation in the wavelength will cause a large error in the absorbance. Most spectrophotometers show a slight fluctuation in the wavelength, so errors in absorbance will be magnified if we select a wavelength such as 500 nm in our preceding example.

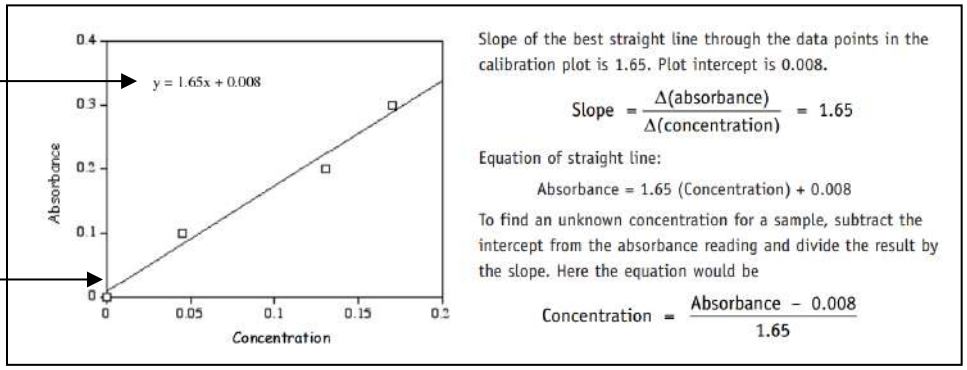
3. Plotting Calibration Graphs

Once we have chosen the correct wavelength, the next step is to construct a **calibration curve** or **calibration plot**. This consists of a plot of absorbance versus concentration for a series of standard solutions whose concentrations are accurately known.

Because calibration curves are used in reading off the unknown concentrations, their accuracy is of absolute importance. Therefore, make the standard solutions as accurately as possible and measure their absorbances carefully. Each standard solution should be prepared in identically the same fashion, the only difference between them being their concentrations.

When drawing the calibration graphs, take care not to lose any of the accuracy of the experimental data by choosing axes that are too small. Choose axes to represent the accuracy possible in reading the instrument. For example, if it is possible to read absorbance correct to the second decimal place, say 0.47, then construct the absorbance axis so that 0.47 can be located accurately on it.

You will find in your experiment that the calibration plot generally does not pass through the origin, but it should be quite close. Thus, the value you see for the intercept (b) in the equation $y = mx + b$ will be small.



The Beer-Lambert Law ($A = \varepsilon \bullet \ell \bullet c$) implies that when concentration is equal to zero (c = 0), absorbance must also be zero (A = 0). In other words, the calibration line must pass through the origin.

A major source of error in spectrophotometric analysis is applying the Beer-Lambert Law at inappropriate concentrations. The Beer-Lambert Law is strictly applicable only for dilute solutions. It becomes less and less accurate as the concentration of the solution increases.

Once you have the calibration curve set up, you can measure the absorbance of any unknown solution at the same wavelength and read off its concentration from the graph or calculate from the slope.

Method:

1. Prepare a dilute sample for each color to be tested using a cuvette and distilled water (approximately 1 drop food coloring to 100 ml distilled water).
2. For each color sample fill a cuvette.
3. Prepare a blank sample cuvette containing distilled water only.
4. Use the color wheel to predict absorbance values for each solution and record your predictions in the table provided.
5. Set up the spectrometer to scan the visible region from 350-800 nm and run each sample at 20nm increments.
6. Once the maximum(s) have been identified, to get a more precise maximum value(s), redo the maximum range(s) at 5nm increments.
7. Enter the data into a spreadsheet and create a graph.
    a. Label the graph with a heading of the color.
    b. Label the x, y-axis (include the units)
    c. Color code the line of the graph to match the color of the solution.
8. On the graph, place an "X" at the maximum(s) and record the wavelength.
9. Print out the spectrum and compare these with your predictions.
10. Pick a color and make a series of dilutions (1/2 concentration and ¼ concentration). Repeat the process of making a spectral profile of that color, for the ½ and ¼ concentrations. Overlay the data onto the original colored graph (full concentration, ½, and ¼ concentration).

Materials:

Chemicals:

- Food coloring samples – Red, yellow, green, blue, orange, black
- De-ionized/distilled water

Apparatus:

- Cuvettes
- Wash bottles
- 100mL graduated cylinder
- 200mL beaker
- Chem-Wipe Tissues

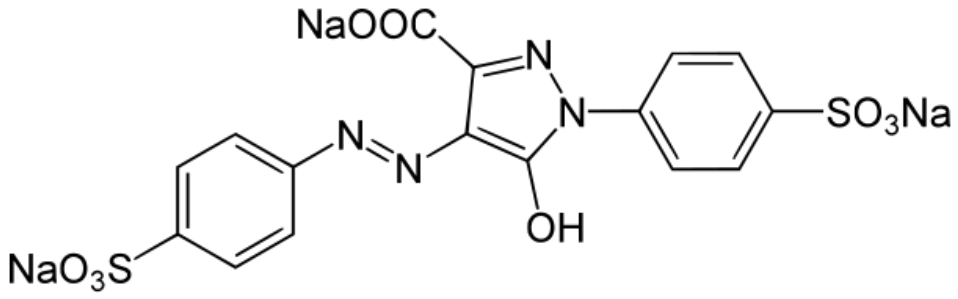
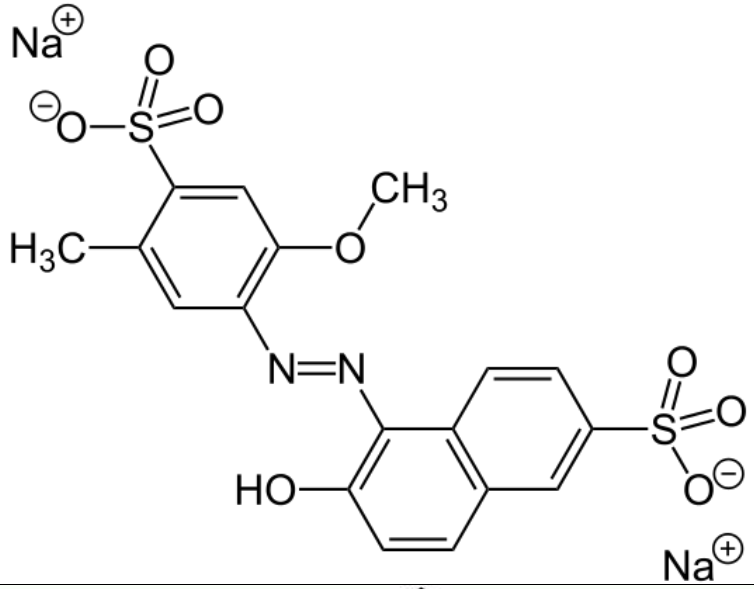
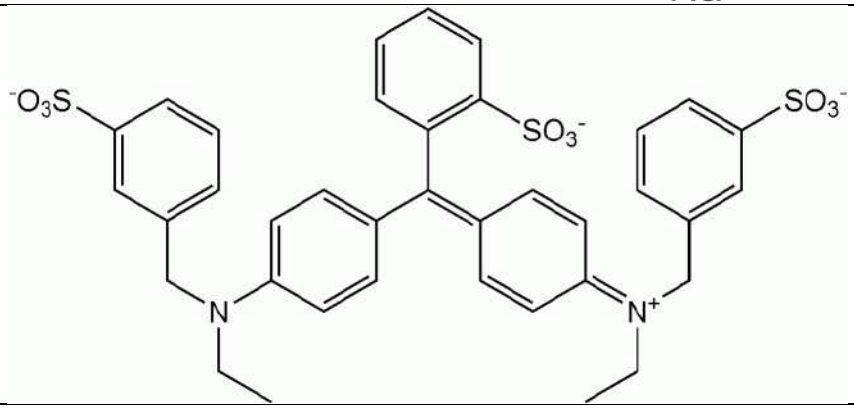
Instrument:

- Spectrometer
- Computer/Chromebook

Setup for laptop:

- Setup a spreadsheet (Excel or Google Sheets)
    - Title of the color you are testing
    - Two columns (x-axis = wavelength (nm), y-axis = Absorbance)

- Measure Absorbances at 20nm increments. When a maximum(s) have been identified, go back and repeat at 5nm increments before and after the maximum(s).

Food dye	Molecular structure
Yellow	
Red	
Blue	

Student Worksheet

Color	Predicted Absorbance Value (nm)	Actual Maximum Absorbance Value(s) (nm)	Notes
Red			
Yellow			
Green			
Blue			
Orange			
Black			

Observations to note:

1. For colors that are made from a mixture of dyes, what is observed of their peaks, in comparison to the original dyes (ie. Comparing orange to red and yellow dyes)?
2. Comparing the full, ½, and ¼ concentration solutions, what is noticed about the peak(s) of the same colored dyes? Do the peak(s) change wavelengths? Measure the heights of each peak and explain the relationship between the peak(s) height(s) to their concentrations.
3. If possible, take a colored solution and shine a light through it that is the same color and its complementary color.
    a. What was the color of the dye used?
    b. What happened to the light of the same color?
    c. What happened to the complementary colored light?
    d. If using a laser, such as a red laser, what type of safety goggles would need to be used?

Results

Color	Predicted Absorbance Value (nm)	Actual Absorbance Value (nm)	Notes
Red	496 – 570	519 & 528	Absorbs Green
Yellow	380 – 450	428	Absorbs Violet
Green	620 – 750	427 & 635	Absorbs Red
Blue	590 – 620	409 & 628	Absorbs Orange
Orange	430 – 480	?	Absorbs Blue
Black	?	519 & 635	Note: This absorbs both in the Red and Green which are directly opposite, solution appears black