Title: UV-Visible Experiment

Alan Yu*, Haroon Chaudhry, Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08901.

Abstract:

This experiment was to test the Beer Lambert's Law and the relationship between absorbance and concentration, molar absorptivity, and path length with the use of UV-Vis. The spectra acquired was used to create calibration curves to determine many of the items stated above. The standards were Cobalt and Chromium and were found to be 0.208M and 0.06M in concentration respectively. In the experiment it was found that both sample serial dilutions had a linear relationship between absorbance and concentration which is to be expected from the Beer Lambert's Law. The equation and regression for Co was found to be $Y = 0.892x + 0.150$ and R^2 is 0.998 and at 561nm is: $Y = 0.450x + 0.308$ and R^2 is 0.980. For Cr it was found to be $Y = 2.68x + 0.117$ and R^2 is 0.992, and at 561nm is: $Y = 6.40x + 0.289$ and R^2 is 0.999. The equations were used to find the molar absorptivity coefficients for both Co and Cr and they were 0.747 $\frac{L}{mol*cm}$ and 5.36 $\frac{L}{mol*cm}$ respectively by dividing the slope by the pathlength being 1.194cm. The unknowns were found to be 0.090M for Cobalt, 0.024M for Chromium, and 0.057M and 0.063M for Cobalt and Chromium mix respectively.

*To whom correspondence should be addressed.

I.   Introduction

We used UV-Vis spectrometry to determine the concentrations of Co and Cr in aqueous solutions and determined the UV-Vis absorption spectra of a bottled sample with color. For my pre-laboratory report, Nghia [1] and the team used UV-Vis to determine color intensity in their probe proposal in determining folic acid in real samples such as drug tablets, human serum, and urine. I also read two additional peer-reviewed journal articles that were published in 2019 and they were summarized together with my pre-laboratory report journal article in Table 1. The first additional article I read was an experiment in determining the concentrations of dissolved organic matter in drinking water sources in the Nanfei River by Zhang [2] and the team. The team determined based on UV-Vis that different seasons, specifically dry to wet season transitions, causes a large difference in concentration of dissolved organic matter. The second addition article that I read was an experiment that determined the conformation of Gal(LTCu) films by Oliveira Neto [3] and the team. They used UV-Vis to determine that this sample of Gal(LTCu) film they prepared acquired a square planar conformation and used it to study vibrational and thermal qualities from this conformation.

Table 1. My refereed journal articles.

Corresponding Author	Date Submitted	Date Accepted	Samples	My reference number
Nguyen Ngoc Nghia	July 15, 2019	September 13, 2019	Folic acid, Rhodamine B in drug tablets, human serum, urine	1
Huan Zhang	July 18, 2019	September 6, 2019	Dissolved organic matter in drinking water sources	2
João G. Oliveira Neto	June 10, 2019	September 27, 2019	Gal(LTCu) films	3

For UV-Vis absorption to occur, the organic molecules require $n \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, and $n \rightarrow \pi^*$ transitions. From page 395-398 of the Textbook, [4] a few terms are defined. A **chromophore** is a group of atoms that allow the electron transitions listed above, and an **auxochrome** is a substituent that contains unshared electron pairs. If an auxochrome is attached to a chromophore, with π electrons, a shift to longer wavelengths is observed. With this, there are two types of wavelength shifts available and the first one is a **bathochromic shift** where the wavelength is shifted to a longer wavelength and the second is a **hypsochromic shift** where the

wavelength is shifted to a shorter wavelength. Figure 5.41, from the Textbook, [4] shows a hypothetical spectrum with example shifts.
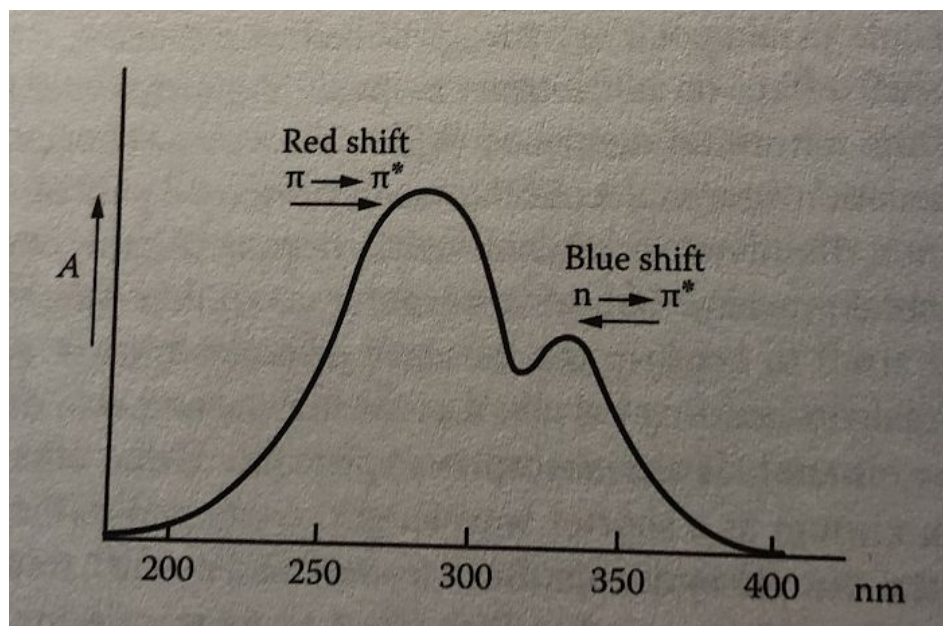

Figure 1. Figure 5.41 [4] from textbook displaying bathochromic, or red shift, and hypsochromic, or blue shift.

There were also a few equations that were used to calculate specific values such as error, concentration, and molar absorptivity. The first equation used was for error propagation:

$$\frac{X}{E} = \sqrt{(\frac{a}{A})^2 + (\frac{b}{B})^2} \qquad (1)$$

The second equation that was used was the standard deviation equation,

$$\sigma = \sqrt{\frac{\sum\limits_{i=1}^{n}(X^*-X_i)^2}{n-1}} \qquad (2)$$

where σ is the standard deviation, X^* is the mean and n is the amount of samples in the test. The third equation that was used was the dilution equation where,

$$C_1 V_1 = C_2 V_2 \qquad (3)$$

C_1 is the initial concentration, V_1 is the initial volume, C_2 is the concentration wanted, and V_2 is the volume for the desired concentration. The fourth equation that was used was the Beer Lambert's Law where,

$$A = Cb\varepsilon \qquad (4)$$

A is the absorbance, b is the path length of the cuvette that had the light shine through, C is the concentration, and ε is the molar absorptivity. A fifth equation is the relationship between energy and wavelength:

$$E = hv = \frac{hc}{\lambda} \tag{5}$$

where E is the energy, h is Planck's constant, v is the frequency, c is the speed of light, and λ is the wavelength. This equation is used to determine the energy in respect to the wavelength where a photon emits.

II. Experimental

Equipment:

Instrumentation: Ocean Optics, Inc. RedTide USB650UV computer-based spectrometer. (Dunedin, Florida), plastic cuvette, glass vials, and Ohaus analytical balance, Item#: PA214, Max Cap: 210g, Made in China.

Chemicals: Chromium (III) nitrate nonahydrate (CAS 13548-38-4), Cobalt (II) nitrate hexahydrate (CAS 10026-22-9), both obtained from Sigma-Aldrich (Saint Louis, Missouri).

Stock solutions of the Cr and Co were prepared by Chemistry 348 staff and data for the solutions are summarized in Table 2. The stock solutions were prepared by weighing the respective solids on the Ohaus analytical balance and then transferring the solids to a 250 mL Class A volumetric flask (Error: ±0.12 mL) and diluting to the mark with high purity deionized distilled water. Table 1 shows the data for the standard preparations.

Table 2. UV-Vis standard stock solution preparations

Standard	Tare Mass (g)	Sample Mass (g)	Concentration (M)	Error (M)
Cr (III)	0.0000	6.0023	0.0600	±0.0003
Co (II)	0.0000	15.1336	0.2080	±0.0001

I used propagation of error to determine the error in the concentration for the stock solutions based on the standards in Table 2 and the Ohaus balance (±0.0001g).

Table 3 summarizes the diluted samples that were prepared using the Ohaus balance.

Table 3. UV-Vis Standard Solutions

Sample	Concentration (M)	Concentration 1 (M)	Concentration 2 (M)	Concentration 3 (M)
Cr (III)	Stock	0.02035	0.03011	0.03908
Co (II)	Stock	0.06879	0.09944	0.1359

I used propagation of error to determine the error in concentration for the first dilution based on the standards in Table 3 and the Ohaus balance that I used that had an uncertainty of 0.0001g. My calculated uncertainty for Co was determined to be ±0.002M and for Cr was determined to be ±0.002M.

We prepared a mixture of Co and Cr concentrations by mixing the stock solutions and the mixture contained 0.12M Co and 0.071M Cr.

Brief Description of Procedure:

There was only one week for the UV-Vis experiment. We took stock standards of Cr(III) and Co(II) and diluted both to become ⅓, ½, and ⅔ of the original concentration as well as creating a mixture of the two standards. These samples, along with the standards, were placed in a UV-Vis spectrometer and their absorbances were collected by the RedTide machine. Three unknowns were also measured in the RedTide machine, one being a Cr(III) unknown in concentration, a Co(II) being unknown in concentration, and a third being a mix of both standards. After acquiring the data for the standards, dilutions, and unknowns, a drink sample was brought in to be measured in the RedTide machine. The sample was Vitamin Water Power-C Dragonfruit flavored water. This was put into the machine and the absorbance was measured to determine the concentration of color inducing ions based on the label on the sample.

III.   Results and Discussion

The absorbance for the different Cr and Co diluted solutions are summarized in Table 4.

Table 4. Cr and Co Absorbance and Concentrations of Standards

Cr	Concentration (M)	Absorbance (561nm)	Absorbance (515nm)
⅓ Dilution	0.02035	0.42	0.17
½ Dilution	0.03011	0.48	0.20
⅔ Dilution	0.03908	0.54	0.22
Co	Concentration (M)	Absorbance (561nm)	Absorbance (515nm)
⅓ Dilution	0.06879	0.34	0.21
½ Dilution	0.09944	0.35	0.24
⅔ Dilution	0.1359	0.37	0.27

From Table 4, I plotted the correlation between absorbance (Y-Axis) and concentration in molarity (X-Axis) for the Co and Cr standards as shown in Figures 2 and 3 respectively.
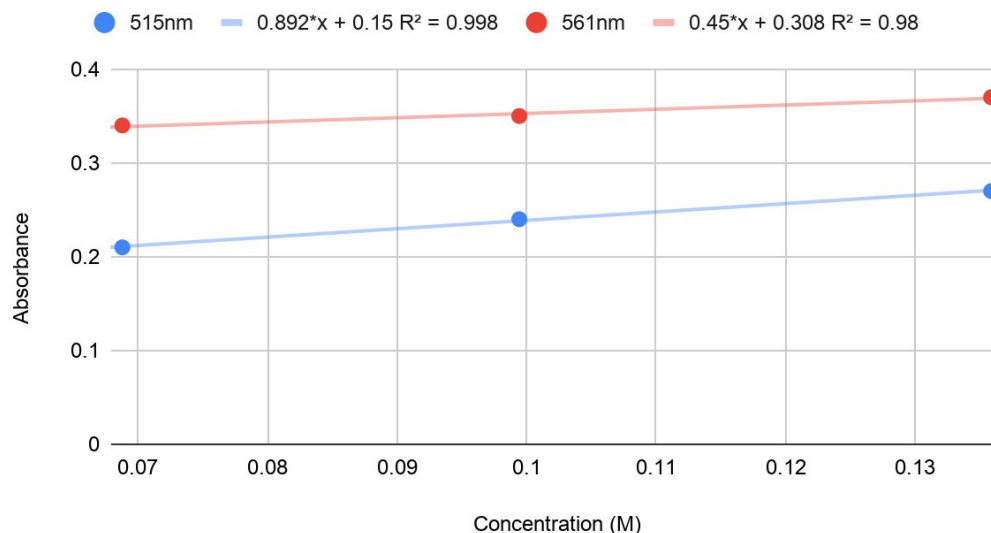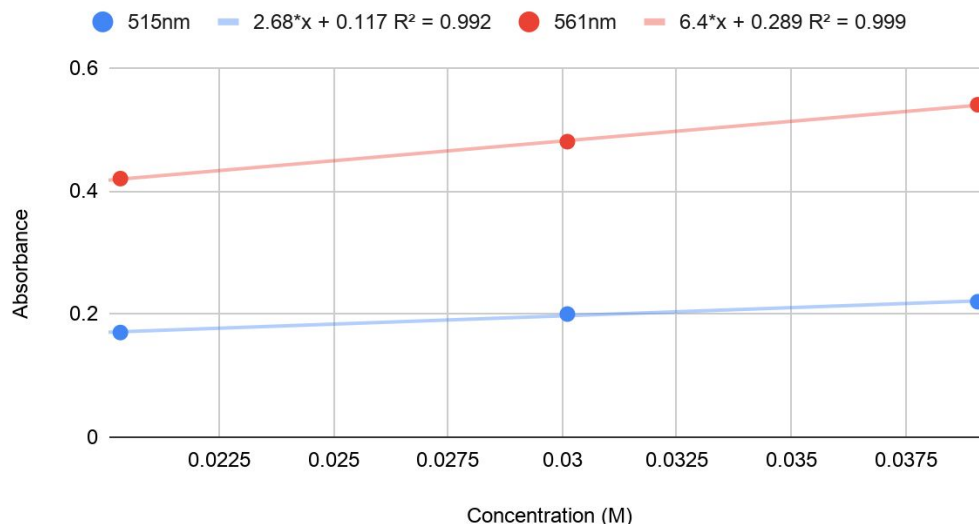
Absorbance vs. Concentration (M) for Co

Figure 2. Plot showing correlation between absorbance and Co concentrations at 515nm and 561nm. Line equation at 515nm is: $Y = 0.892x + 0.150$ and R^2 is 0.998. Line equation at 561nm is: $Y = 0.450x + 0.308$ and R^2 is 0.980.

Absorbance vs Concentration (M) for Cr

Figure 3. Plot showing correlation between absorbance and Cr concentrations at 515nm and 561nm. Line equation at 515nm is: $Y = 2.68x + 0.117$ and R^2 is 0.992. Line equation at 561nm is: $Y = 6.40x + 0.289$ and R^2 is 0.999.

From the equation of the line for the Co plot shown in Figure 2, the slope for 515nm is 0.747 $\frac{L}{mol*cm}$. From the equation of the line for the Cr plot shown in Figure 3, the slope for 561nm is 5.36 $\frac{L}{mol*cm}$.

Given these wavelengths, the corresponding equations can be set to be:
$$A_{515} = 0.892C_{Co} + 0.15$$
$$A_{561} = 0.450C_{Co} + 0.308$$
$$A_{515} = 2.68C_{Cr} + 0.117$$
$$A_{561} = 6.40C_{Cr} + 0.289$$
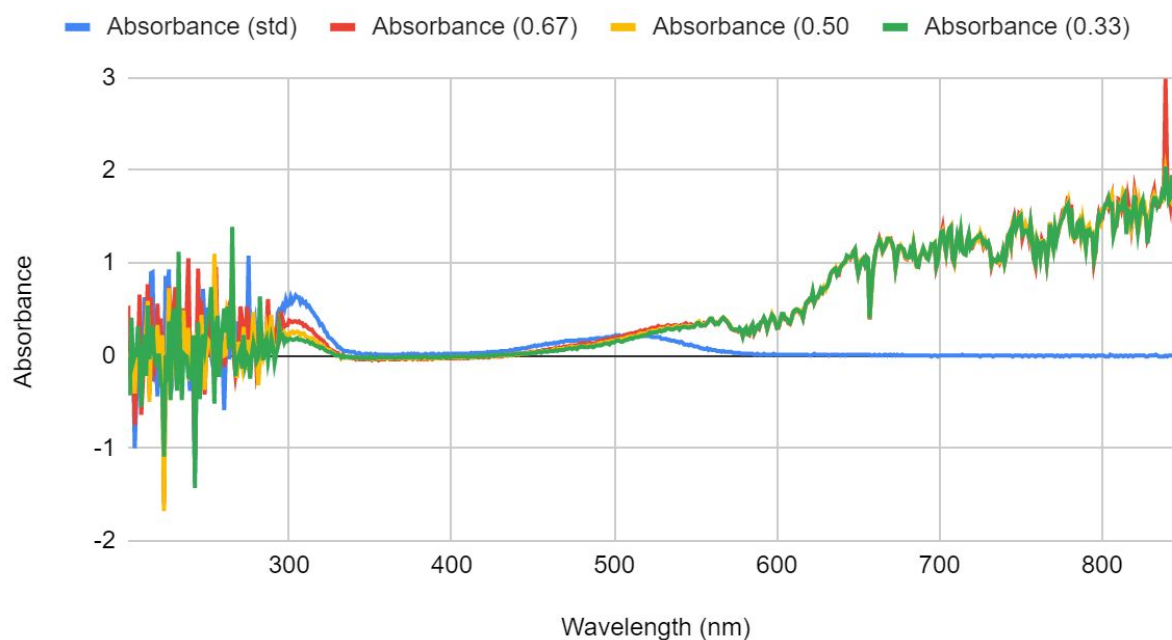
Figure 4. Absorption spectra of the Co standards
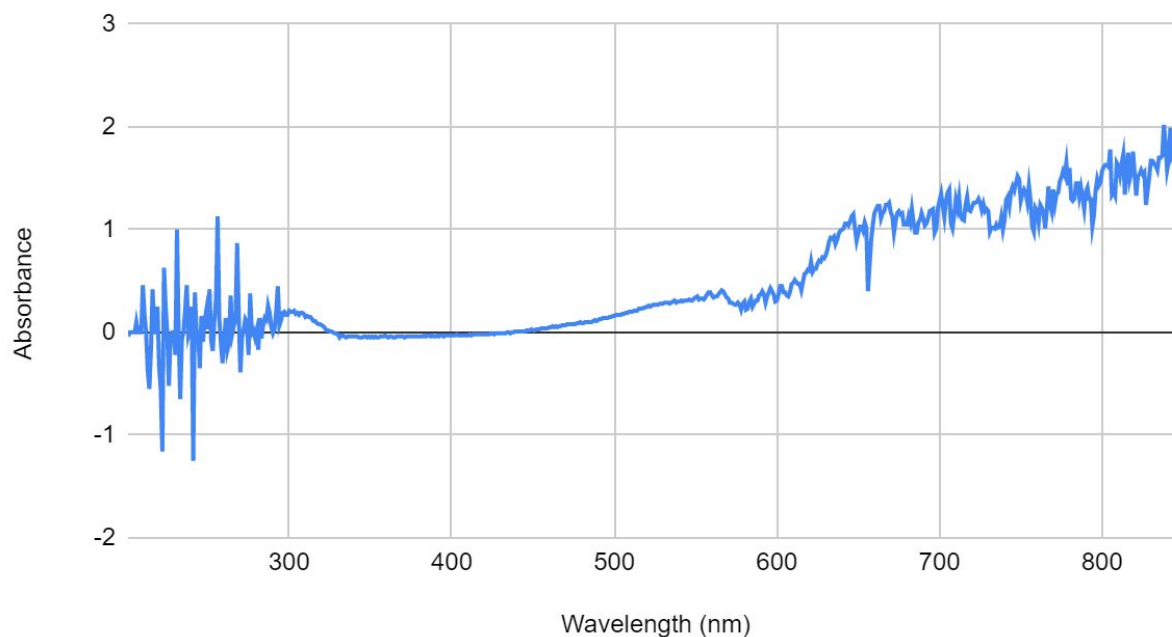
Figure 5. Absorption spectra of Unknown Co sample A-1.
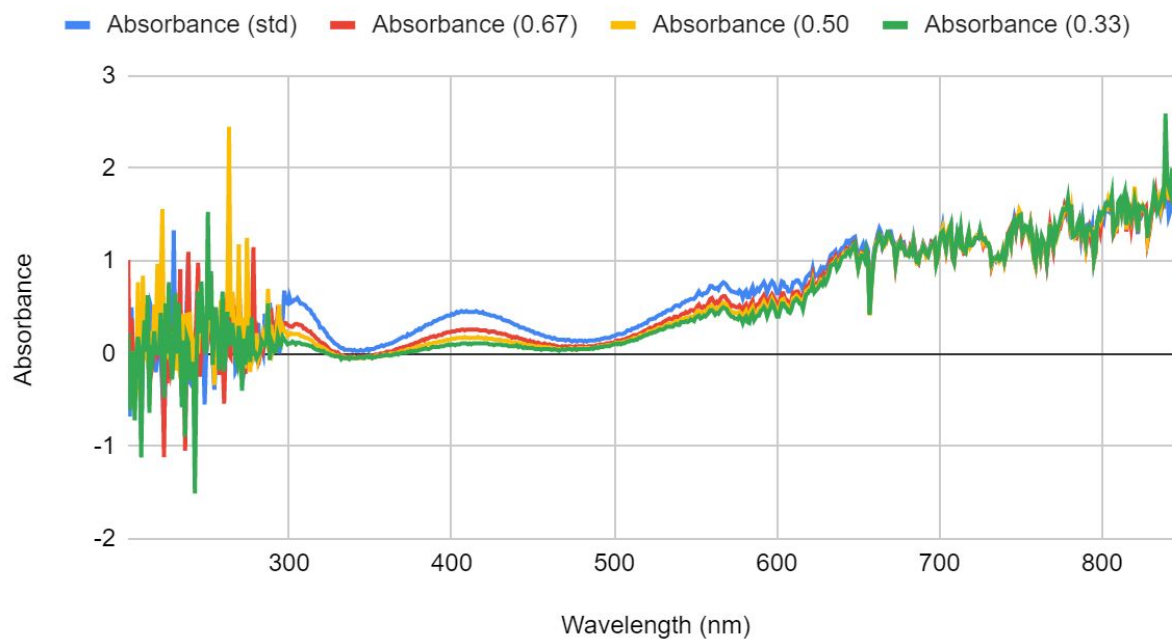
Figure 6. Absorption spectra of the Cr standards.
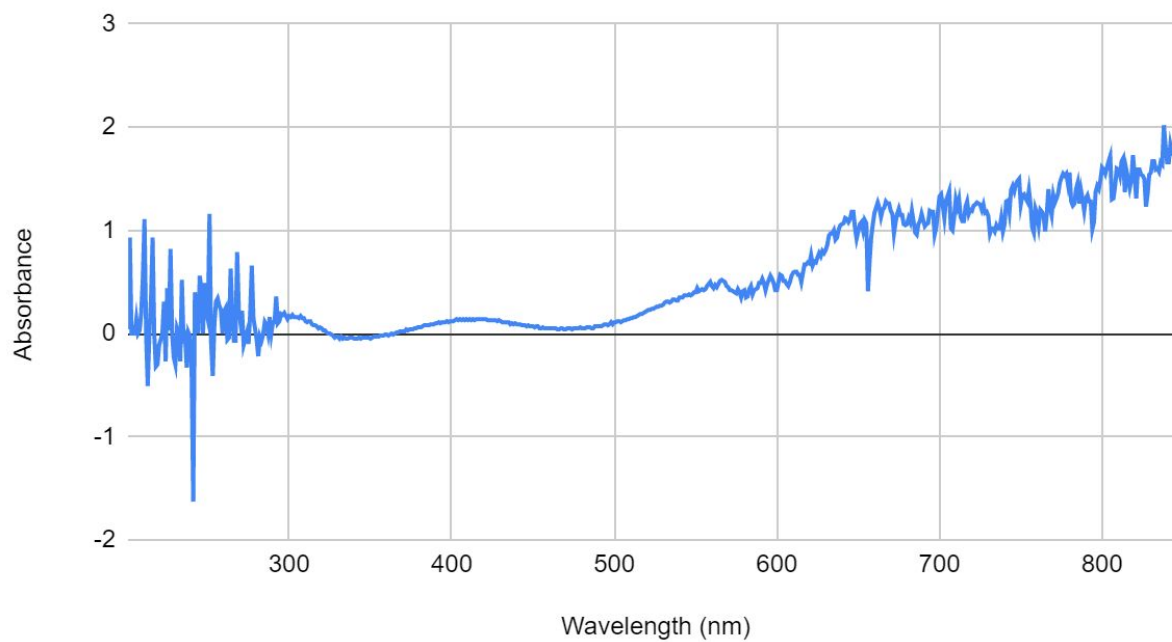
Figure 7. Absorption spectra of Unknown Cr sample A-1.
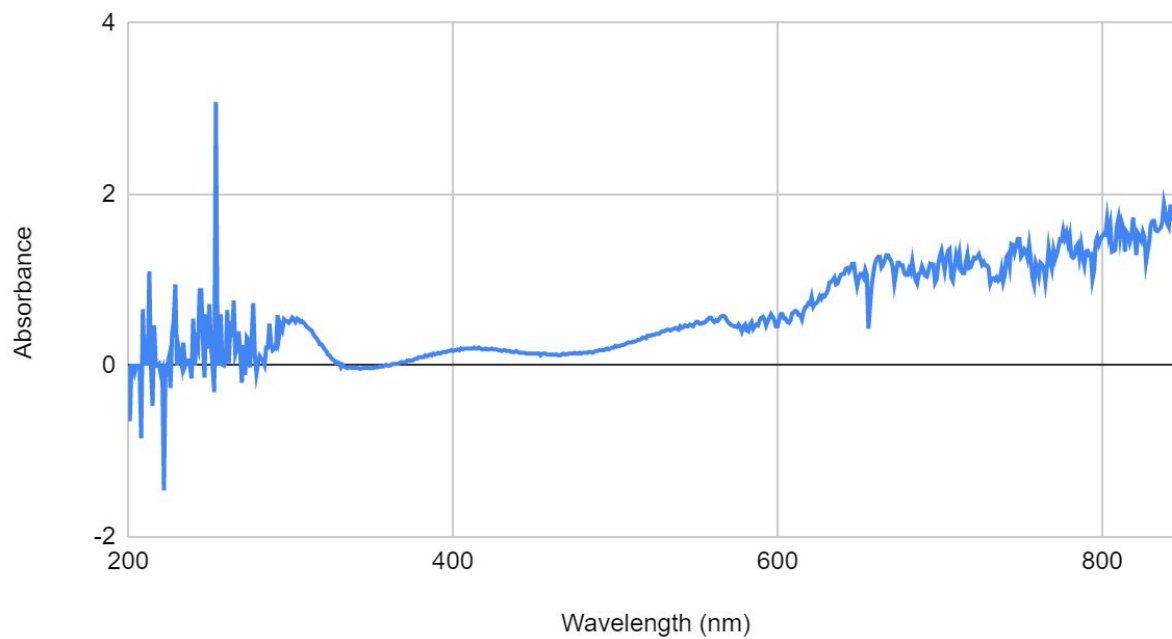
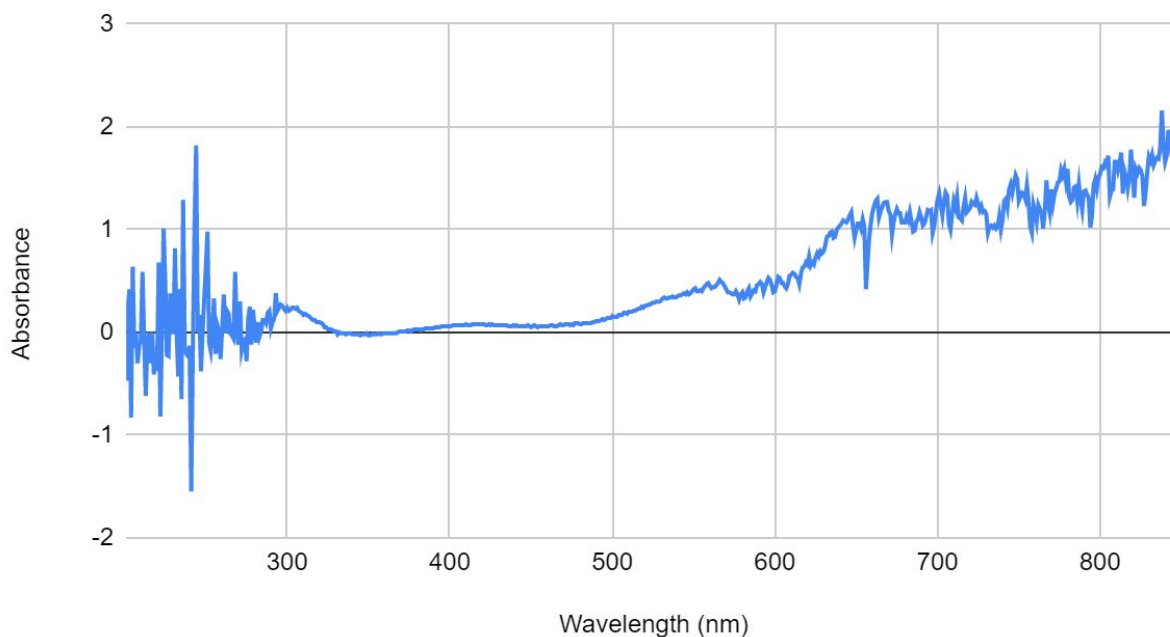Figure 8. Absorption spectra of Co and Cr mixture.

Figure 9. Absorption spectra of Unknown Co and Cr mixture B-1.

Table 5. Concentrations of Co and Cr in the Unknown Solutions.

Unknown ID	Co Concentration (M)	Cr Concentration (M)
Co A-1	0.090	0
Cr A-1	0	0.024
Co and Cr B-1	0.057	0.063

For Cobalt at 515nm and Chromium at 561nm:

$$0.23 = 0.892C_{Co} + 0.15$$
$$C_{Co} = 0.0897$$
$$0.44 = 6.40C_{Cr} + 0.289$$
$$C_{Cr} = 0.0236$$

For a mix of two different samples, the sum of all the corresponding equations are found and used to solve for each respective value using linear algebra omitting constants to follow Beer-Lambert's Law:

$$A_{515} = 0.892C_{Co} + 2.68C_{Cr}$$
$$A_{561} = 0.450C_{Co} + 6.40C_{Cr}$$

Setting the equations equal to the absorbances:
$$0.22 = 0.892C_{Co} + 2.68C_{Cr}$$
$$0.43 = 0.450C_{Co} + 6.40C_{Cr}$$
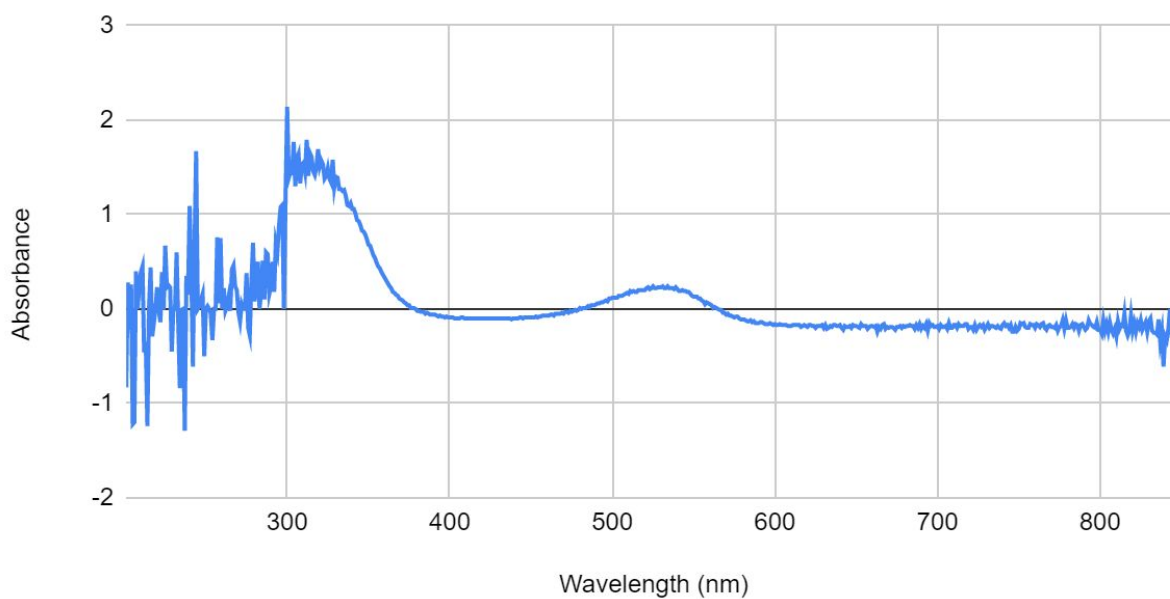Setup the equations to be reduced row echelon form and the values are acquired.

Figure 10. Absorption spectra of Vitamin Water power-c dragonfruit sample

Table 6. Absorption Maximum of Bottled Sample

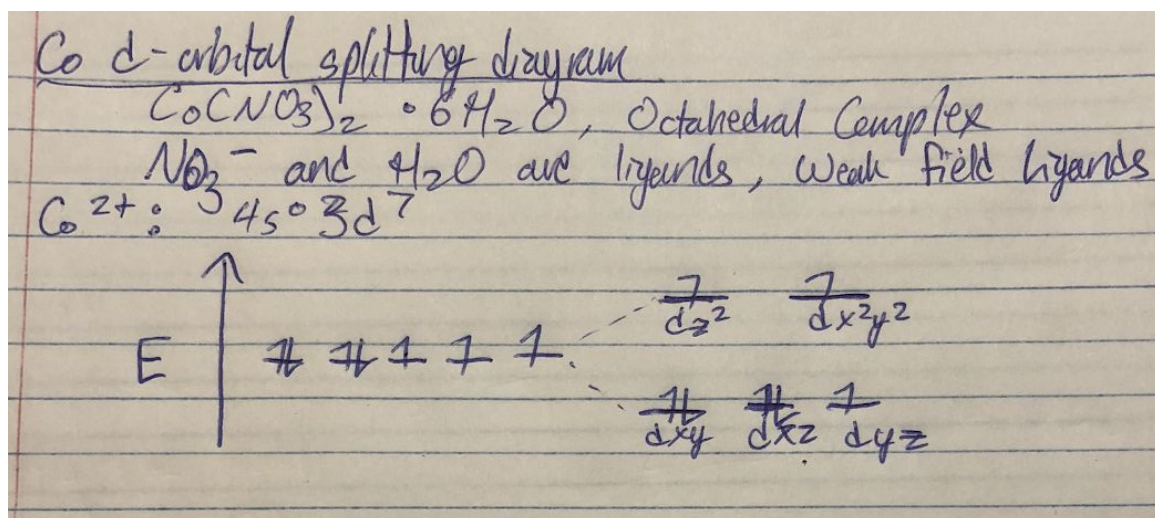| Sample	Absorption Max (305nm)	Absorption Max (530nm)
Vitamin Water	1.3	0.23

Co d-orbital splitting diagram
$CoCNO_3)_2 \cdot 6H_2O$, Octahedral Complex
NO_3^- and H_2O are ligands, Weak field ligands
$Co^{2+}: 4s^0 3d^7$

$$E \uparrow \quad \updownarrow \updownarrow \uparrow \uparrow \uparrow$$

$\overline{\uparrow}$ d_{z^2} $\overline{\uparrow}$ $d_{x^2y^2}$

$\updownarrow$ d_{xy} $\updownarrow$ d_{xz} $\uparrow$ d_{yz}

Figure 11. Co d-orbital splitting diagram with the H_2O and NO_3^- as weak field ligands.

Cr d-orbital splitting diagram
$CrCNO_3)_3 \cdot 9H_2O$
NO_3^- and H_2O are ligands, weak field ligands
$Cr^{3+}: 4s^0 3d^3$

$$E \uparrow \quad \uparrow \uparrow \uparrow - -$$

$\overline{}$ d_{z^2} $\overline{}$ $d_{x^2y^2}$

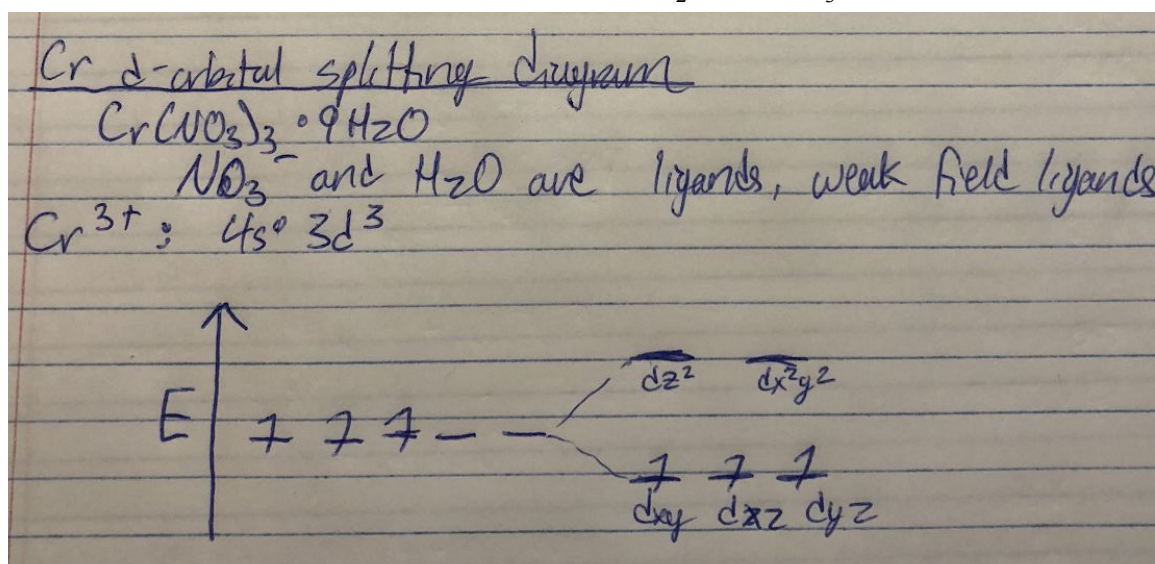$\uparrow$ d_{xy} $\uparrow$ d_{xz} $\uparrow$ d_{yz}

Figure 12. Cr d-orbital splitting diagram with the H_2O and NO_3^- as weak field ligands.

Vitamin C	150%
Pantothenic Acid	100%
Vitamin B6	100%
Vitamin B12	100%
Zinc	25%

Not a significant source of saturated fat, trans fat, cholesterol, dietary fiber, vitamin D, calcium, iron and potassium.

Caffeine Content: N/A

INGREDIENTS

reverse osmosis water, crystalline fructose, less than 1% of: cane sugar, vitamins C, B5, B6, B12 (ascorbic acid, calcium pantothenate, pyridoxine hydrochloride, cyanocobalamin), electrolytes (calcium and magnesium lactate and potassium phosphate), taurine, citric acid, vegetable juices (for color), natural flavors, zinc gluconate, chromium polynicotinate.

Figure 13. Vitamin Water ingredients list from the Coca-Cola website.

IV.   Conclusions

In the experiment, UV-Vis spectroscopy was used to determine the differences within the Cobalt(II) and Chromium(III) absorbance spectra. For both samples, a calibration curve was created from ⅓, ½, and ⅔ dilutions of the stock sample. The equations that came from the calibration curves at 515nm and 561nm specifically gave a system of equations that allowed us to find unknown concentrations in the samples. The unknowns were found to be 0.090M for Cobalt, 0.024 for Chromium, and 0.057 and 0.063 for Cobalt and Chromium mix respectively.

The diagrams of the splitting diagrams of the Cobalt and Chromium complexes can be used to describe the specifics of why the ions have different colors although the same valence orbital. Since the human eye perceives colors that are complementary instead of what they absorb, the one for Cobalt absorbed at the 515nm which is a greenish blue range in absorption, but the complementary comes out as a pink. For Chromium, the absorption was around 561nm where it absorbed yellow, but was perceived to be the complementary which was violet.

These colors are observed because of the d-splitting orbitals that both Cobalt and Chromium has. Since both of the samples used had octahedral coordination complex geometry, they had roughly the save crystal field splitting energy. Within the diagrams from Figure 11 and Figure 12, Cobalt could be seen to have all orbitals partially filled while Chromium has only

three orbitals partially filled rather than all of it partially filled. Since Cobalt has a higher effective nuclear charge, and the higher orbitals are partially filled, promoting an electron to a higher energy level in Cobalt requires more energy which yielded a shorter wavelength to be emitted. For Chromium this would be the opposite where it requires less energy to promote an electron into a non filled orbital and Chromium also has a smaller effective nuclear charge which yields the longer wavelength to be emitted.

Given the ingredients label for the Vitamin Water power-c dragonfruit flavored sample seen in Figure 13, the label states that there are vegetable juices for color, but another possibility that could have caused color would be the chromium complex that is in very small amounts within the drink.

V. References

1. Nguyen Ngoc Nghia, Bui The Huy, and Yong-Ill Lee, Paper-based colorimetric probe for highly sensitive detection of folic acid based on open-ring form amplification of Rhodamine B derivative. Journal of Industrial and Engineering Chemistry, 1226-086X, 2019.
2. Huan Zhang, Kangping Cui, Zhi Guo, Xiaoyang Li, Juan Chan, Zhaoguo Qi, and Siyuan Xu, Spatiotemporal variations of spectral characteristics of dissoled organic matter in river flowing into a key drinking water source in China. Science of the Total Environment, Volume 700, 134360 (January 2020).
3. João G. Oliveira Neto, Lincoln A. Cavalcante, Eduardo S. Gomes, Adenilson O. Dos Santos, Francisco F Sousa, Fernando Mendes, and Ana Angélica M Macêdo, Crystalline Films of L-Threonine Complexed with Copper (II) Dispersed in a Galactomannan Solution: A Structural, Vibrational, and Thermal Study. Polymer Engineering and Science, Volume 59, (2019).
4. Undergraduate Instrumental Analysis, James W. Robinson, Eileen M. Skelly Frame, George M. Frame II, CRC Press, 2014, 7th edn.