Title: Fall 2019 Chemistry 348 Fluorescence Experiment

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Abstract: This lab had two weeks to it. The first week was determining if the standards were fluorescent and if they were, to determine the emissions from the six standards that were given. Rhodamin 6G was clear as a given standard that had 527nm excitation and 551nm emission. Rhodamin B was pink as a given standard that had 548nm excitation and 566nm emission. Quinline Yellow was yellow as a given standard that had 528nm excitation and 562nm emission, Methyl Violet 2B was light violet that had 243nm excitation and 383nm emission. Fluorescein was a lime standard that had 240nm excitation and 514 emission, and Riboflaven was clearish yellow that had a 261nm excitation and 547nm emission. For the second week, two of the six standards that showed the best emissions to create calibration curves that can be used to determine the concentration of the standard solutions given. The concentration of Methyl Violet 2B was found to be 0.08284 g/g and Rhodamin 6G was found to be 0.03597 g/g. The propagation of error for Methyl Violet 2B was 0.01034 g/g and for Rhodamin 6G was 0.01053 g/g. The second part of week two was finding spectra of two flavor infused bottled drinks, which were Vitamin Water and Powerade respectively. Vitamin Water had 293nm excitation and 264nm emission while Powerade had 290nm excitation and 390nm emission.

Keywords: Fluorescent, emissions, excitation, calibration curve, propagation of error.

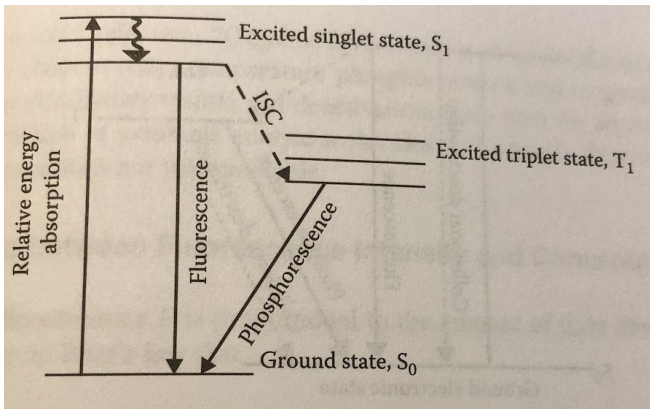
I. Introduction:

We used fluorescence spectrometry to determine the wavelengths and emissions of six standard compounds that were in the lab as well as two different samples of flavor infused bottled drinks that we brought in. From my pre-laboratory report, Govindaraju [1] and the team experimented with fluorescence to enhance gold nanoclusters in order to detect epinephrine in candidates sensitively. I also read two additional peer-reviewed journal articles that were published in 2019 and they are summarized together with my pre-laboratory report journal article in Table 1. The first additional article I read was an experiment done on pistachios by Qifang Wu and their team. [2] They used fluorescence to determine aflatoxin content within the nuts. The second additional article I read as an experiment done on Candida, commensal fungi to humans, by Weslley F. Oliveira and their team. [3] Their team used quantum dots conjugated with fluorescent nanoprobes to profile the species of Candida they were experimenting on.

Table 1: My refereed journal articles

Corresponding Author	**Date Submitted**	**Date Accepted**	**Samples**	**My reference number**
Govindaraju	May 30, 2019	August 29, 2019	AuNCs-Epinephrine NP	1
Wu, Qifang	March 20, 2019	September 6, 2019	Pistachios	2
Oliveira, Weslley F.	July 24, 2019	September 6, 2019	Candida	3

When a compound is exposed to UV light, or black light, and creates a visible shine in the dark, the compound fluoresces. A compound fluoresces when a molecule absorbs a photon and and has an electron that is elevated from the ground state into an excited vibrational state that is a higher electronic state. After being in the higher electronic state, the electron that was excited will quickly vibrate some energy off and release the rest as a photon as the electron recovers back to the ground state. From Figure 5.49 from the textbook [4], a diagram is given for an electron that is excited and degrades back to the ground state.



The textbook also provides equations of the relationship of fluorescence intensity and concentration. The first equation [4] is

$$\frac{I_1}{I_0} = e^{-abc} \quad (1)$$

where I_0 is the transmitted intensity, I_1 is the incident intensity, and a is the absorptivity. The textbook does some arithmetic where the final equation [4] resulted to

$$F = I_0(1 - e^{-abc})\Phi \quad (2)$$

where F is the fluorescence intensity, and Φ is the quantum yield or quantum efficiency. This explains that the fluorescence intensity is directly proportional to the concentration of analyte, quantum efficiency, intensity of incident radiation, and absorptivity of analyte. The propagation of error equation was used to determine the error in mass knowing the balance's uncertainty. The equation [4] is

$$\Delta\left(\frac{A}{B}\right) = \frac{A}{B}\sqrt{\left(\frac{a}{A}\right)^2 + \left(\frac{b}{B}\right)^2} \quad (3)$$

where a is the uncertainty and A is the value acquired and so forth for every other sample to be calculated.

II. Experimental:

Equipment:

Horiba Scientific Aqualog Spectrofluorometer
OHaus Electronic Balance, Pioneer, Item# PA214, 210g max
Finnpipette: 20-200µL, Thermo, CH14459, 4500
Quartz Cuvette
25mL stoppered volumetric flask,
Plastic bulb pipette
4 dram black-capped vials
50µL pipette
Reagents: high purity water, ethanol (spectro grade)

Standards: See Table 2 below.

Table 2: Standards

ID	Compound	Mass (g)	Mass + ETOH (g)
3	Rhodamin 6G	0.00742	10.16924
4	Rhodamin B	0.00641	10.77730
5	Quinline Yellow	0.00820	10.07690
7	Methyl Violet 2B	0.00072	9.21196

ID	Compound	Mass (g)	Mass + ETOH (g)
8	Fluorescein	0.00880	10.07750
9	Riboflaven	0.05680	102.71990

The above samples were diluted (#D) by taking 0.1 mL of each and adding to 10-mL of water. Sample 8 was diluted by taking 0.6 mL of standard and adding to 10-mL of water, determined by mass. The uncertainty of the balance is 0.0001 g.

Table 3: Diluted fluorescent samples for calibration curve

Standard	Conc. 1 (g/g) (50µL dilution)	Conc. 2 (g/g) (100µL dilution)	Conc. 3 (g/g) (150µL dilution)	Conc. 4 (g/g) (200µL dilution)	Conc. 5 (g/g) (no dilution, Lab Made)
Methyl Violet 2B	0.01159	0.02292	0.03435	0.04495	0.08284
Rhodamin 6G	0.01132	0.02277	0.03358	0.04574	0.03597

I used propagation of error to determine the error in concentration for the first dilution based on the standards in Table 2 and the OHaus balance that I used that had an uncertainty of 0.0001g. My calculated uncertainty was determined to be 0.01034 g/g for Methyl Violet 2B and 0.01053 g/g for Rhodamin 6G.

Procedure Description:

There were two weeks allocated for this lab procedure. Week one was the introduction of the Spectrofluorometer. First a blank of ethanol and high purity water was taken to calibrate against the machine when conducting the standard measurements. After the blanks were acquired, each standard was ran through the Spectrofluorometer which included absorbance spectra graphs, waterfall plots, contour maps, etc.. The graphs and data that were collected were also corrected through the Aqualog program. After correcting, peak absorbances were collected of each standard to run for Week two. Week two required two standards that had great contour maps as well as each person bring in a sample of flavor-infused drinks. Both standards that were chosen had increments of 50µL starting from 50, and ending at 200. This was ran inside the Spectrofluorometer and resulted in a calibration curve. The two bottled samples were diluted and ran within the Spectrofluorometer and had the data corrected as well.

III. Results and Discussion:

Table 4: Summary of Excitation and Emission Wavelengths

ID	Compound	Color	Excitation (nm)	Emission (mm)
3	Rhodamin 6G	Clear	527	551

ID	Compound	Color	Excitation (nm)	Emission (mm)
4	Rhodamin B	Pink	548	566
5	Quinline Yellow	Yellow	528	562
7	Methyl Violet 2B	Light Violet	243	383
8	Fluorescein	Lime	240	514
9	Riboflaven	Clearish Yellow	261	547

Table 5: Summary of Excitation and Emission Wavelengths of bottled water

ID	Bottled Water	Color	Excitation (nm)	Emission (mm)
1	Vitamin Water Zero	Cloudy White	293	394
2	Powerade	Dark Blue	290	390

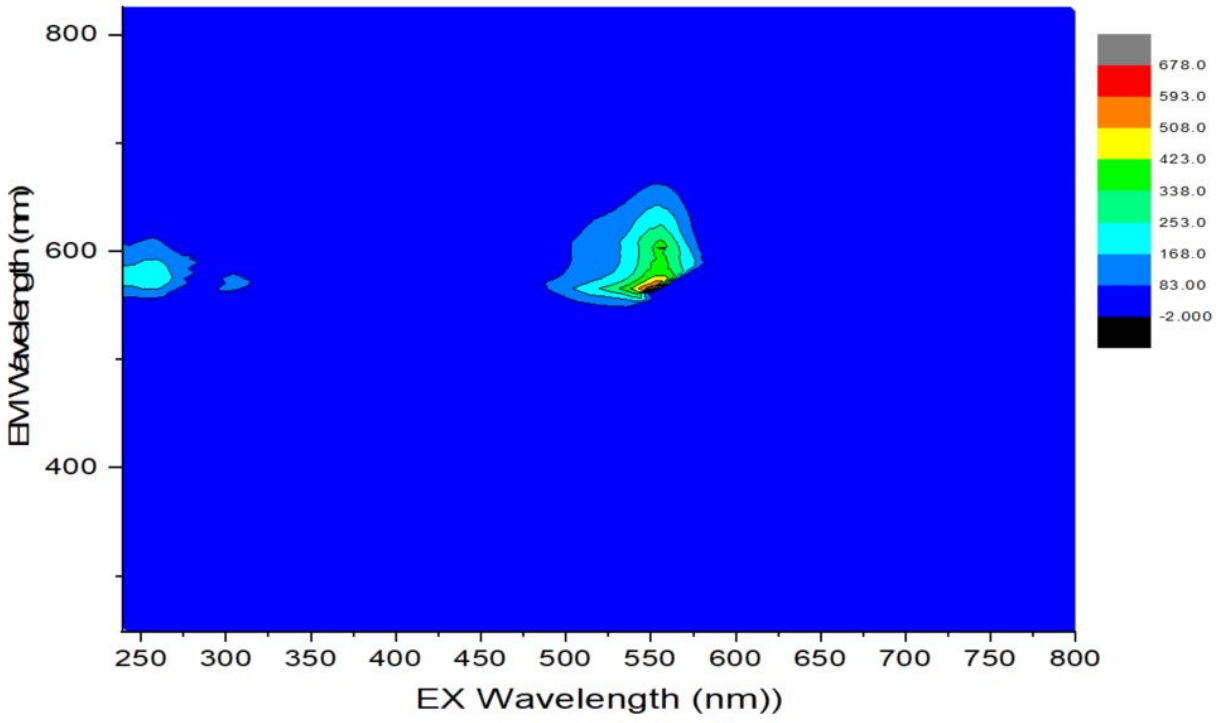


Figure 1. EEM of Rhodamin B

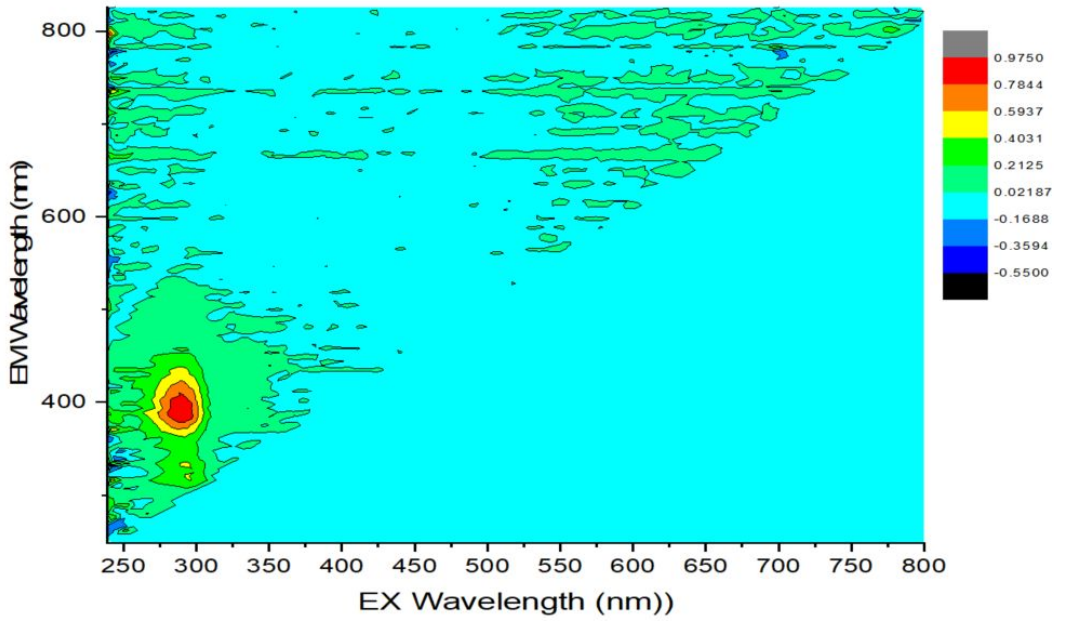


Figure 2. EEM of bottled Powerade

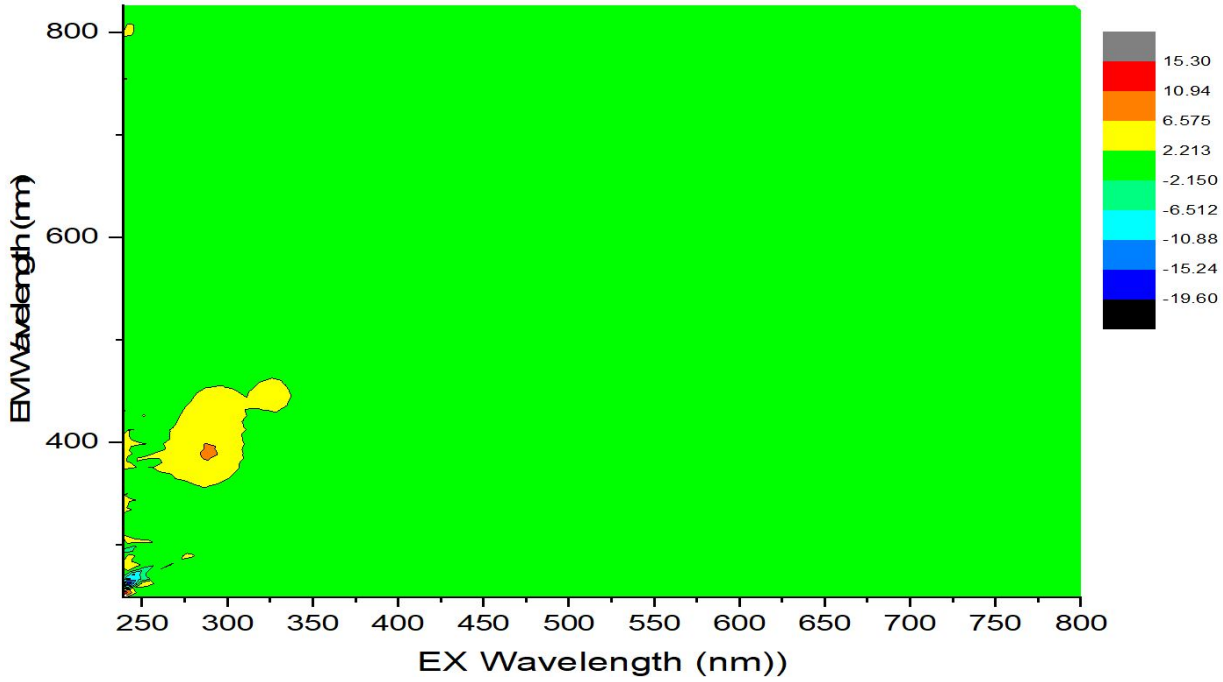


Figure 3. EEM of bottled Vitamin Water

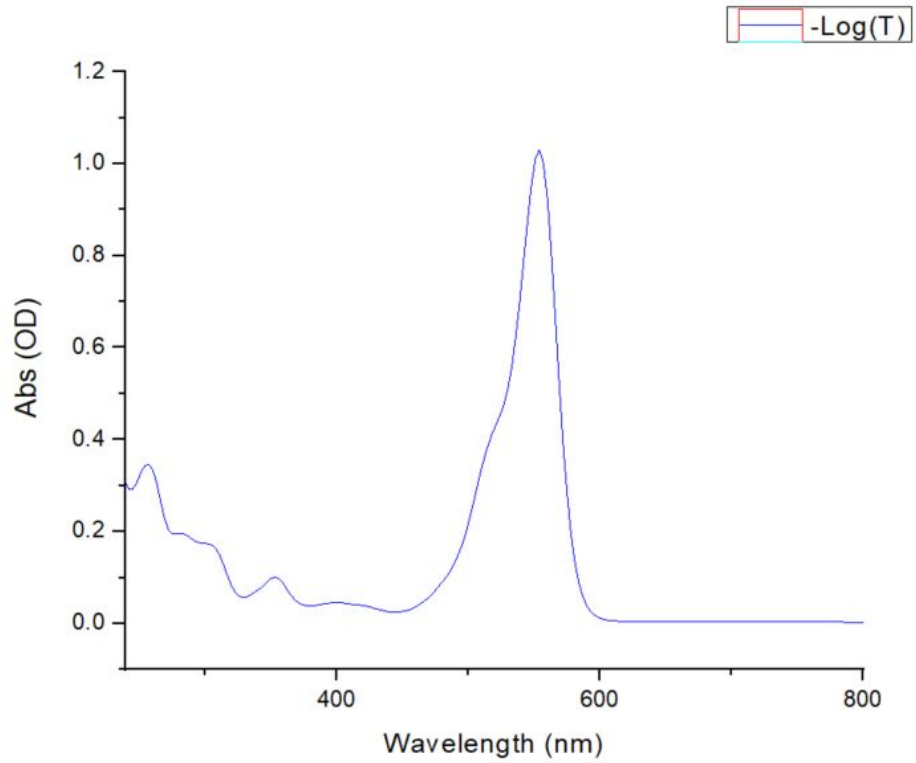


Figure 4. Absorbance spectra of Rhodamin B

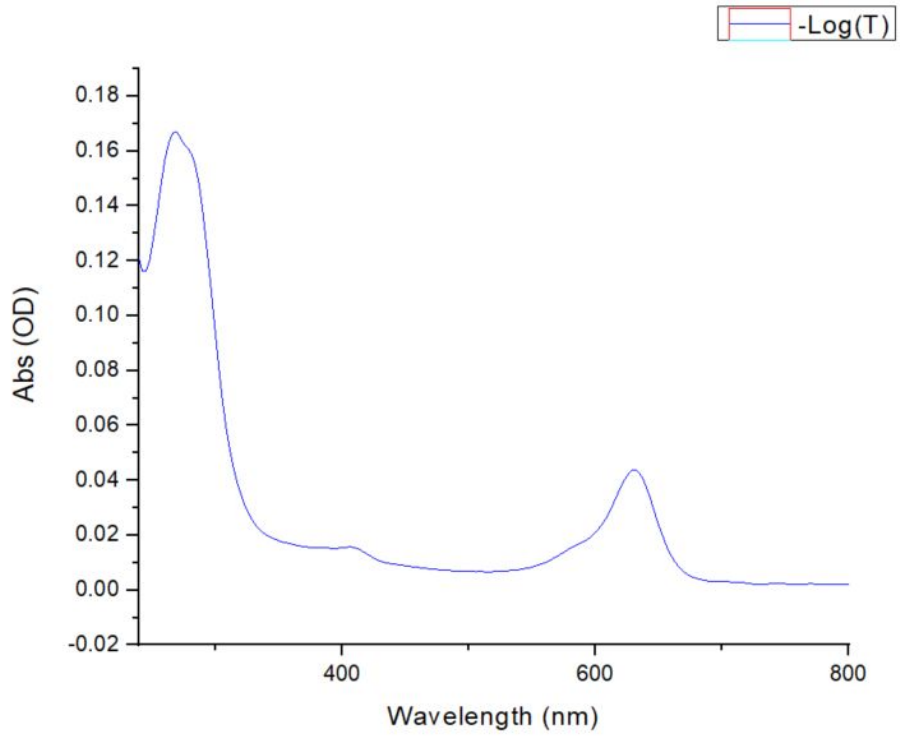


Figure 5. Absorbance spectra of bottled Powerade

Figure 6. Absorbance spectra of bottled Vitamin Water

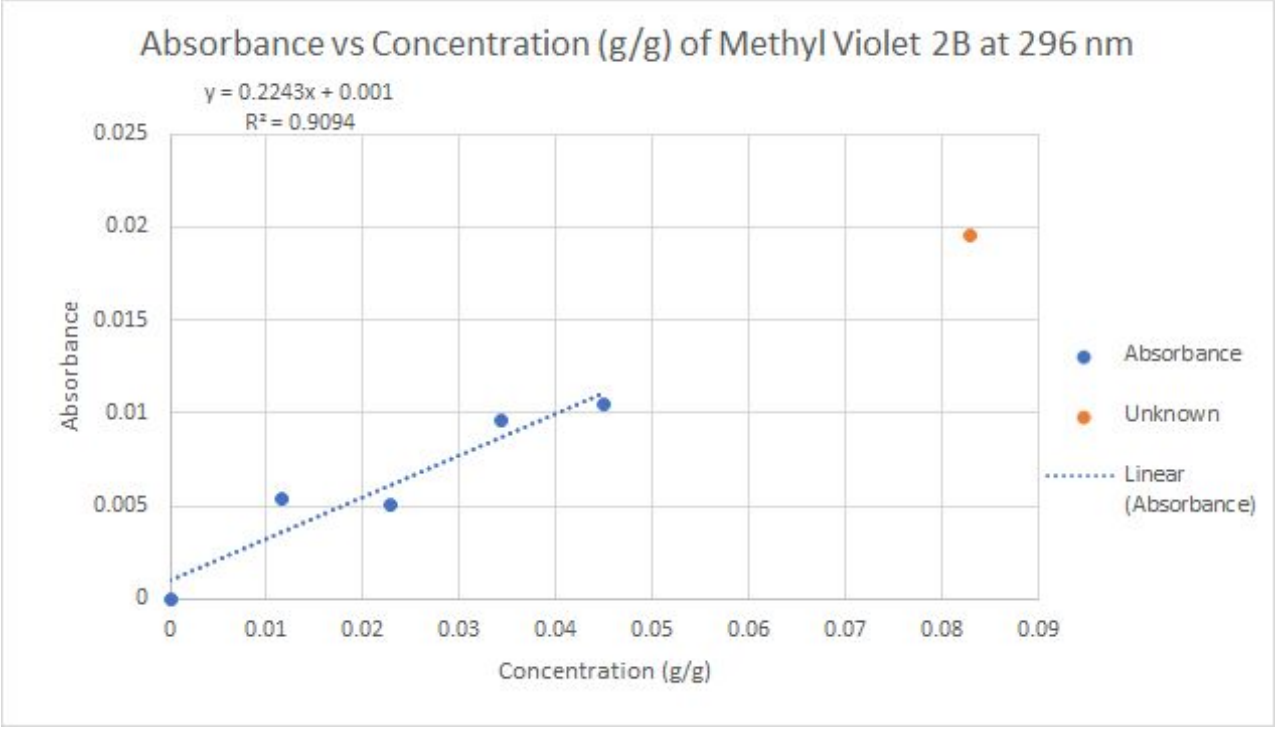


Figure 7. Calibration plot of Methyl Violet 2B at 296nm, where the slope represents the molar absorptivity of the sample.

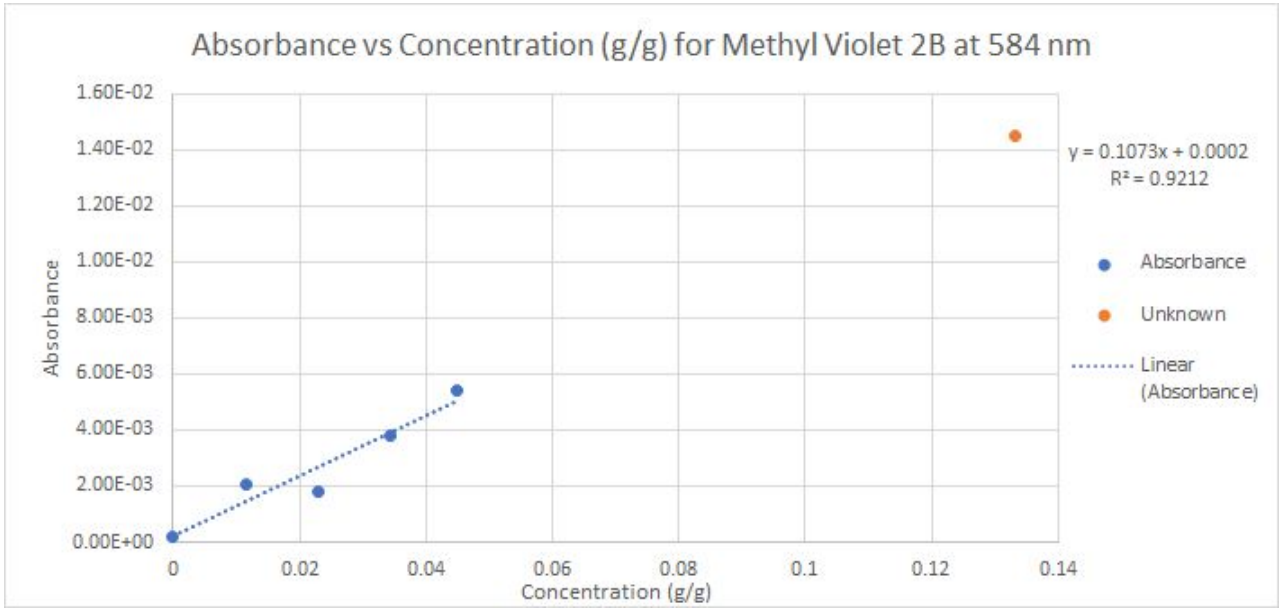


Figure 8. Calibration plot of Methyl Violet 2B at 584nm, where the slope represents the molar absorptivity of the sample.

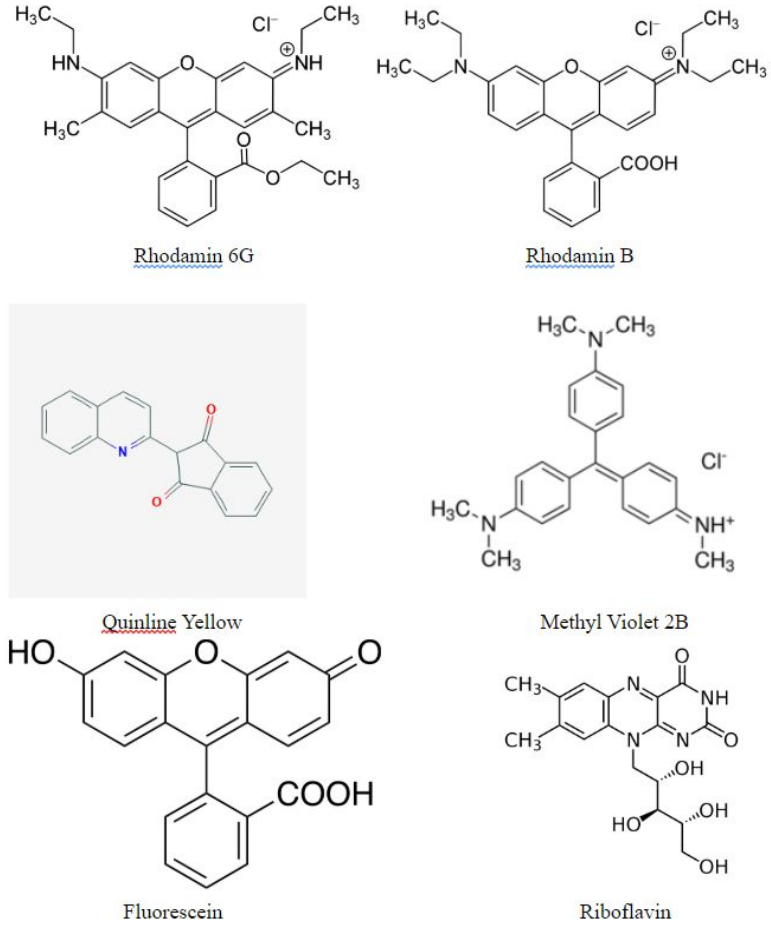


Figure 9. Molecular structures of the compounds that fluoresce

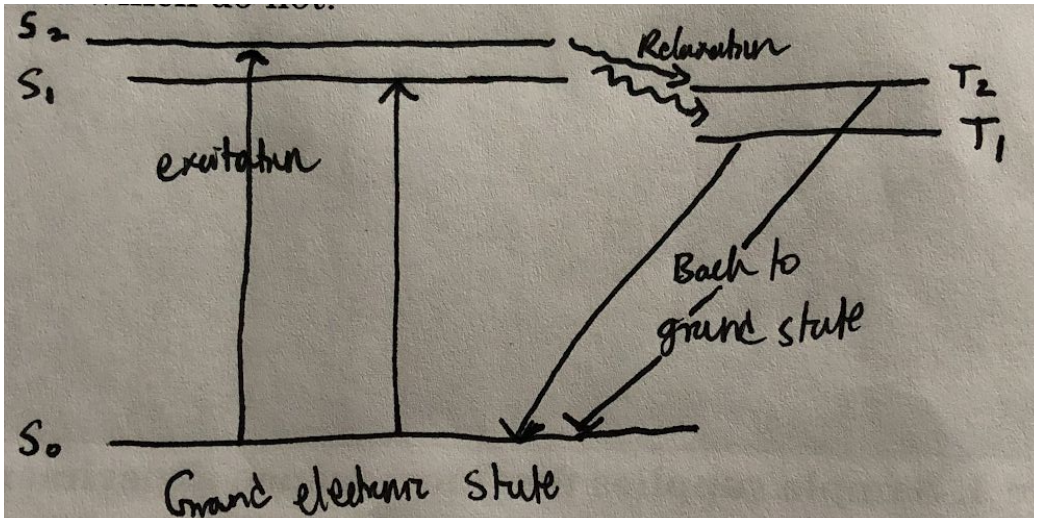


Figure 10. Energy level diagram of Rhodamin B

IV. Conclusions:

Based on the data, all compounds showed some sense of fluorescence. Comparing the fluorescence to the chemical structures of each compound that fluoresced, it can be deducted that the compounds that fluoresced had conjugation available to them when light was there to excite the molecules. The more pi bonds that are available for conjugation yields a higher emission wavelength since the amount of energy is lowered to excite electrons from the ground state due to the multiple resonance structures that are available for the conjugated molecules. Using Table 4, the yellow-red solutions that was observed had around a 520nm emission while the blue-violet solutions had about a 400nm emission. From Figure 5 and Figure 6, the R^2 value for each figure respectively was .9094 and .9212. This shows that for methyl violet 2b, about 90% of the time, the compound has a close to linear relationship between concentration, absorbance, excitation and emission wavelengths. A propagation of error was calculated for the calibration curves, although only methyl violet 2B was the best shown. The calculations yielded 0.01034 g/g for Methyl Violet 2B and 0.01053 g/g for Rhodamin 6G. Using the bottled samples, the Powerade and Vitamin Water showed some absorbance in their spectrums. The diluted Powerade showed some absorbance near the 630nm area while the diluted Vitamin Water had a decreasing curve that didn't show any peaks in the absorbance graph. For bottled samples, the ones that had coloring can be concluded that there are fluorescent dyes in the drinks to make them the color they are, while for non-colored drinks there can be dyes in the drinks dependent on manufacturer. An error that messed up the data collection could have been the instrument or the preparation of each dilution. Since each standard was prepared in high purity water for this batch, it could have been different from last batch where ethanol was used instead as the solvent.

V. Miscellaneous Questions from Procedure:

1. Samples are entered from low to high concentrations because of the linear relationship from Beer's law that this experiment was determining. Having from high to low can also show the linear relationship, but will show a decreasing relationship. Having the curve from low to high helps determine whether or not Beer's law is true for the proportional relationship of concentration and absorbance.

VI. References:

1. Saravanan Govindaraju, Ankireddy Seshadri Reddy, Jongsung Kim, and Kyusik Yun, Sensitive detection of epinephrine in human serum via fluorescence enhancement of gold nanoclusters. Applied Surface Science 298 (2019) 143837.
2. Qifang Wu and Huirong Xu, Design and development of an on-line fluorescence spectroscopy system for detection of aflatoxin in pistachio nuts. Postharvest BIology and Technology, 159 (January 2020) 111016.
3. Weslley F. Oliveira, Mariana P. Cabrera, Natalia R.M. Santos, Thiago H. Napoleao, Patricia M.G. Paiva, Rejane P. Neves, Marcia V. Silva, Beate S. Santos, Luana C.B.B. Coelho, Paulo E. Cabral Filho, Adriana Fontes, Maria T.S. Correia, Evaluating glucose and mannose profiles in Candida species using quantum dots conjugated with Cramoll lectin as fluorescent nanoprobes. Microbiological Research, 230 (January 2020) 126330.
4. Undergraduate Instrumental Analysis, James W. Robinson, Eileen M. Skelly Frame, George M. Frame II, CRC Press, 2014, 7th edn.