Spectrophotometric Analysis of Mixtures: Simultaneous Determination of Two Dyes in Solution

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1 Abstract

In this experiment, we created a set of 8 concentrations of 2 dyes, then used a spectrophotometer to calculate the absorbance of the dyes with respect to both concentration and wavelength. Our ultimate goal was to calculate the concentrations of each dye in a solution containing a mixture of both of them. By observing the absorption curve of the unknown, we were able to solve the system of equations

$$\left.\begin{aligned} A_1 &= \epsilon_R^1 b C_R + \epsilon_Y^1 b C_Y \text{ (at } \lambda_1\text{)} \\ A_2 &= \epsilon_R^2 b C_R + \epsilon_Y^2 b C_Y \text{ (at } \lambda_2\text{)} \end{aligned}\right\} \tag{1}$$

for C_R and C_Y, the concentrations of the red and yellow dyes in the unknown, where ϵ_A^X is the coefficient of extinction for a dye A at wavelength X, b is the diameter of the cuvette used to make the measurement, and λ_1 and λ_2 are two different wavelengths, selected to have the greatest differences in absorbance between the two dyes. Using data from the 8 samples of varying concentration (see figures 1 - 6 on pages 6 - 8), we were able to find concentrations for red and yellow dye of $19.4925\frac{mg}{L}$ and $37.7455\frac{mg}{L}$ respectively for our dye sample, and $21.1340\frac{mg}{L}$ and $12.6937\frac{mg}{L}$ respectively for our Gatorade sample. While our measurements could be subject to several errors (including improper calibration of our spectrophotometer, inaccurate solution preparation, or dilution due to reuse of a small number of cuvettes for all of our trials), our data follows a meaningful trend and our calculation for the concentrations of our unknowns is within reasonable bounds.

2 Introduction

The ultimate purpose of this experiment was the practical application of the Beer-Lambert law of absorption to calculate the ratio of red to yellow dye in an unknown orange sample, and, more practically, the ratio of dyes in a sample of Orange^{TM}Gatorade^{TM}.

A spectrophotometer fires beams of light of varying wavelength through a sample, and records a logarithmic ratio of transmission of light, in arbitrary Absorbance Units. The Beer-Lambert law states that the absorbance (a logarithmic ratio of transmittivity) of a sample is directly proportional to the concentration of a sample, holding the length of the path through the sample constant, so it is possible to extrapolate for additional absorbance factors using this data — or to calculate the concentrations of two component dyes, because the sums of their absorbances at a specific wavelength equals the absorbance of the mixture at a specific wavelength.

Before the experiment, we hypothesized that while our first sample, a straight mixture of red and yellow dyes, could be accurately broken down into red and yellow components, the second sample (of Gatorade^{TM}) would not be so easily represented, due to the presence of other dissolved compounds and miscellaneous impurities.

3 Methods

We began our experiment by preparing a set of 8 different concentrations of both red and yellow dye, in a spectrum ranging from $5\frac{mg}{L} - 40\frac{mg}{L}$ of dye. We were given samples of dye of a concentration $50\frac{mg}{L}$, and diluted it with water in appropriate ratios to create the spectrum of dyes we required. While we originally planned to use a serial dilution to create these dyes, a lack of concentrated dye meant that we had to prepare each sample individually. We created the first sample using 1 mL of dye in 9 mL of water, the second using 2 mL of dye in 8 mL of water, and so on, producing eight 10 mL samples that we stored in centrifuge tubes (for lack of a better disposable containing apparatus). Next, we used a water 'blank' to calibrate our spectrophotometer. We then proceeded to pour our samples into cuvettes and calculate the absorption spectrum for each of our sample tubes. We used a Vernier^{TM}VIS-NIR (VISible/Near Infra-Red) Spectrometer to analyze and collect our data.

4 Data and Calculations

By using the best-fit data lines in Figures 5 and 6 and setting them in the form of Equation 1, we can see that $\epsilon_R^1 b = 0.0160$, $\epsilon_Y^1 b = 0.0341$, $\epsilon_R^2 b = 0.0351$, and $\epsilon_Y^2 b = 0.0023$. We can plug the concentration spectrum into this equation, and get the predicted absorbance values, and use the difference between the predicted and absolute values (the residuals) to calculate the 95% confidence interval:

First we find the standard deviation of the residuals using the equation for standard deviation $\sigma = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$, or realistically by using a graphing calculator because who has that kind of time. For $\epsilon_R^1 b$ this value is approximately 0.01245. Because we are calculating a 95% confidence interval for what we are assuming is a normal distribution, 5% of the population is outside the curve, half on each side. We can calculate invnorm(.025) on a graphing calculator to determine the number of standard deviations below the mean this boundary is (or use invnorm(.975) to find the same number of standard deviations above the mean). For a 95% interval, this number is approximately ± 1.95996. This means that the uncertainty is $\pm(1.95996 \times 0.01245) = \pm 0.02440$ and the 95% confidence interval for $\epsilon_R^1 b = 0.0160 \pm 0.02440 = [-0.0084, 0.0404]$. Extending this to the remaining extinction constants ϵ, we can produce this table:

Extinction Constant	Value	Confidence Interval
$\epsilon_R^1 b$	0.0160	[-0.0084,0.0404]
$\epsilon_Y^1 b$	0.0341	[-0.0911,0.1593]
$\epsilon_R^2 b$	0.0160	[-0.0109,0.0155]
$\epsilon_Y^2 b$	0.0160	[-0.0911,0.1214]

Looking at the orange dye sample (figure 3) first, we can see that the absorbance A_1 at λ_1 (423.8 nm)=1.599 AU, as seen in Figure 5. Similarly, the absorbance A_2 at λ_2 (501.3 nm)=0.771 AU. Note that these wavelengths correlate with peaks of the individual dyes' absorption spectra (Figures 1- 2) so as to maximize the difference in the absorbances of the two dyes at that wavelength. This allows us to set up our system of equations:

$$\left.\begin{aligned} 1.599 &= 0.0160C_R + 0.0341C_Y \text{ (at 423.8 nm)} \\ 0.576 &= 0.0351C_R + 0.0023C_Y \text{ (at 501.3 nm)} \end{aligned}\right\} \tag{2}$$

Solving this system of equations yields $C_R \approx 13.7607, C_Y \approx 40.4349$. If we like, we can also plug in our 95% confidence intervals for our extinction constants, we can find 95% confidence intervals for $C_R \approx [3.9961, 23.5253]$ and $C_Y \approx [13.7486, 67.1212]$.

We can set up a similar system of equations for our Gatorade$^{\text{TM}}$sample:

$$\left.\begin{aligned} 0.771 &= 0.0160C_R + 0.0341C_Y \text{ (at 423.8 nm)} \\ 0.771 &= 0.0351C_R + 0.0023C_Y \text{ (at 501.3 nm)} \end{aligned}\right\} \tag{3}$$

Which produces $C_R \approx 21.1340, C_Y \approx 12.6937$. The 95% confidence intervals here are $C_R \approx [10.165, 32.103]$ and $C_Y \approx [3.7532, 21.6342]$.

5 Discussion

In the end, we successfully calculated values for the concentrations of red and yellow dye in both Gatorade$^{\text{TM}}$and our unknown sample. While the variance on these samples is extremely high (because of the very small sample size) the results are both self-consistent and realistic. While the samples can be pruned from results that would skew the sample data, this should generally only be done if a sample fails the Grubbs Test for Outliers (which none of the data points we have do). The absorbance values we have adhere very closely to those predicted by the Beer's law, which states that absorbance is directly related to the concentration of a sample; on a related note, because transmittance has an inverse logarithmic relationship with absorbance, this is why we cannot use transmittance in our calculations (at least not without some very messy arithmetic). One interesting point to note is that the absorption spectrum of Gatorade$^{\text{TM}}$(Figure 4) has very broad, ill-defined peaks compared to the unknown dye sample — perhaps even better described as a plateau. This strongly supports our hypothesis that the Gatorade$^{\text{TM}}$would not be easily represented merely as the sums of red and yellow dyes, due to a large number of obfuscating impurities in the sample. Our calculations can only be assumed to be accurate if the sample consists of only water, and red and yellow dye — something the Gatorade$^{\text{TM}}$does not.

6 Conclusion

During this experiment, we were able to predict the dye concentrations in a sample of orange dye as well as in a sample of Orange$^{\text{TM}}$Gatorade$^{\text{TM}}$. While our results were both relatively precise and accurate, there was a much larger margin of error than we would have liked due to the minute sample size (only 8 trials). To help minimize this margin of error, it would be best to increase the number of concentrations of dyes used to calculate the extinction constant for the Beer-Lambert law. It may be feasible to concentrate these new trials

around the component concentrations we calculated for the unknowns, so as to better represent the unknown samples.

However, there were many possible sources for error that could have occurred throughout the lab. We may have failed to correctly calibrate our spectrophotometer, which could cause systemic error in either the positive or the negative direction. We could have also cross-contaminated our samples of reference dye by failing to properly clean the cuvettes used in the spectrophotometric analysis, which would most likely skew the absorbance values downward, because we analyzed our samples from least to greatest concentration. Finally, there could be some amount of random human error when we were creating our reference samples, because we did so by taking samples of concentrated dye and mixing them with water using transfer pipettes; if we were to measure any of the samples improperly, our concentrations would be off and so would our absorbances.

However, our data seems to correlate relatively well and especially our absorbance spectra (Figs. 1- 2) seem to have few, if any outliers. Our absorption plot of our Unknown (Fig. 3) is similarly uniform, and while our Gatorade^{TM}(Fig. 4) has much more uneven 'plateauing', this only corroborates our inital hypothesis that the Gatorade^{TM}would have an uneven spectrophotometric spectra due to the additional solutes in it (electrolytes — they're what plants crave!)

References

[1] UC Berkeley bSpace: *Spectrophotometric Analysis of Mixtures: Simultaneous Determination of Two Dyes in Solution.* https://bspace.berkeley.edu/access/content/group/4cc7b769-c78a-4044-9771-d138014adc8d/Lab

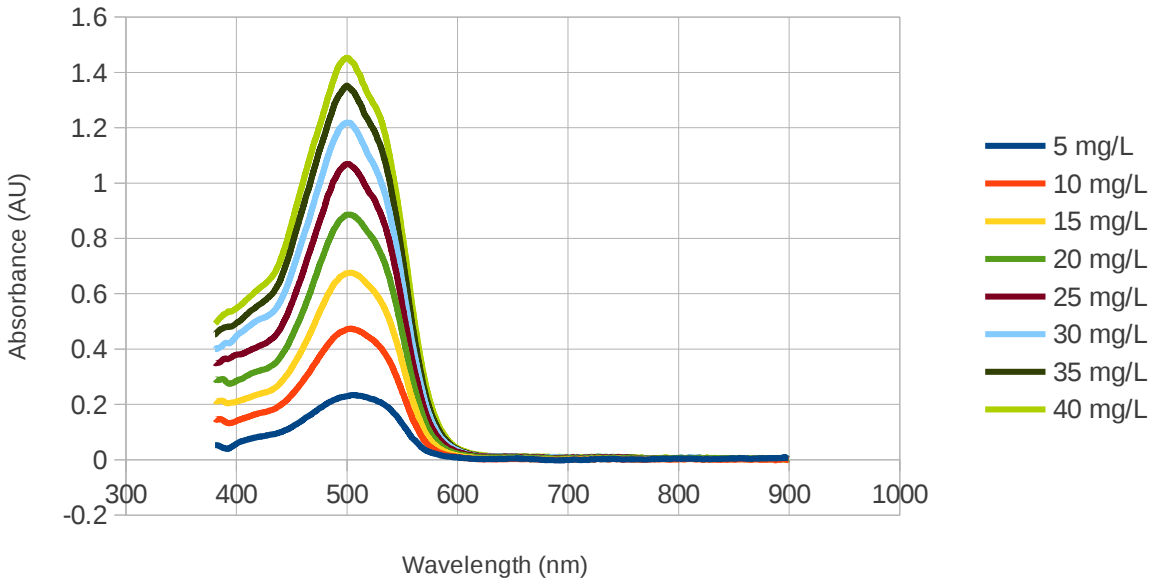

Figure 1: Absorbance vs. Wavelength for Red Dyes

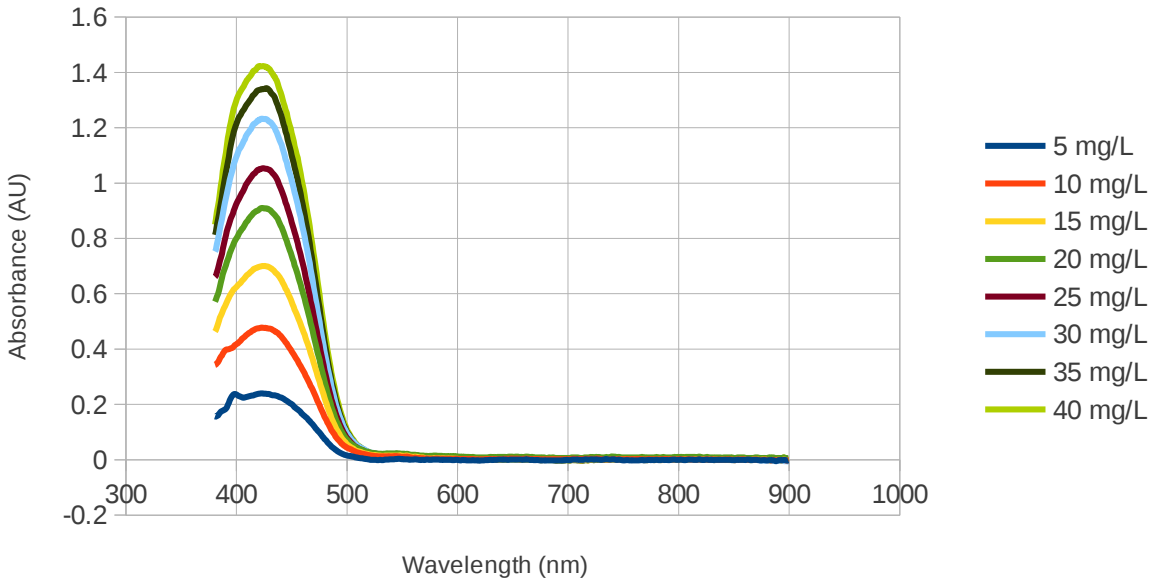

Figure 2: Absorbance vs. Wavelength for Yellow Dyes

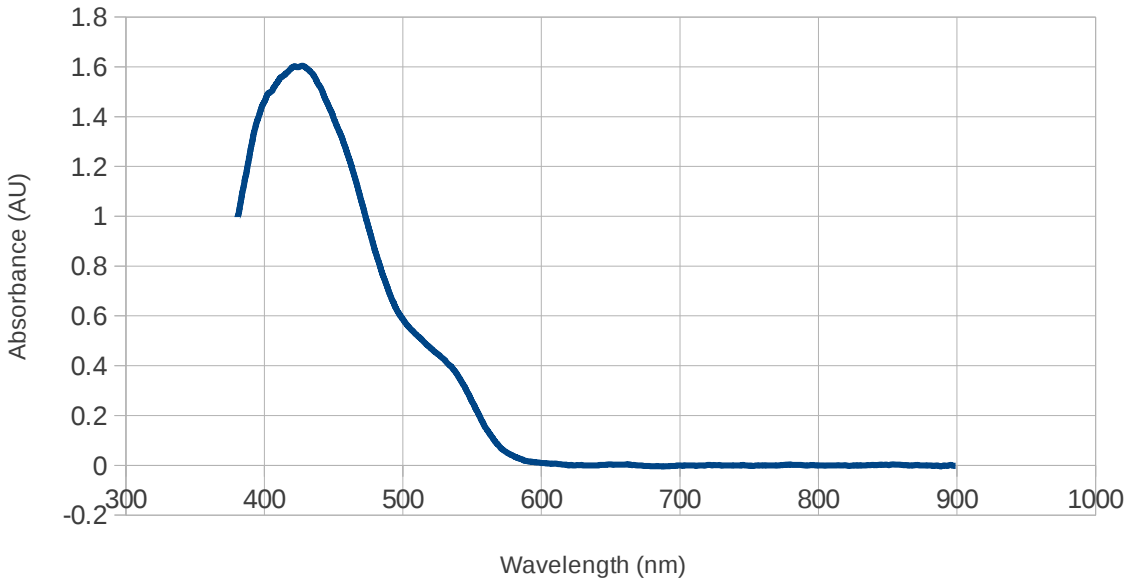

Figure 3: Absorbance vs. Wavelength for Unknown Dye Sample

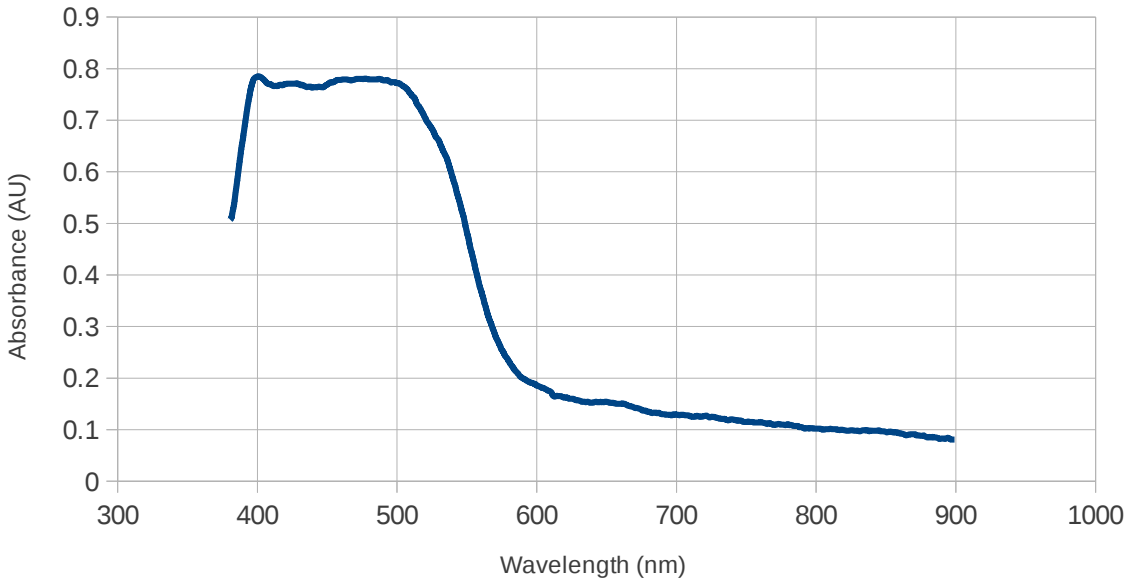

Figure 4: Absorbance vs. Wavelength for Gatorade^{TM}Sample

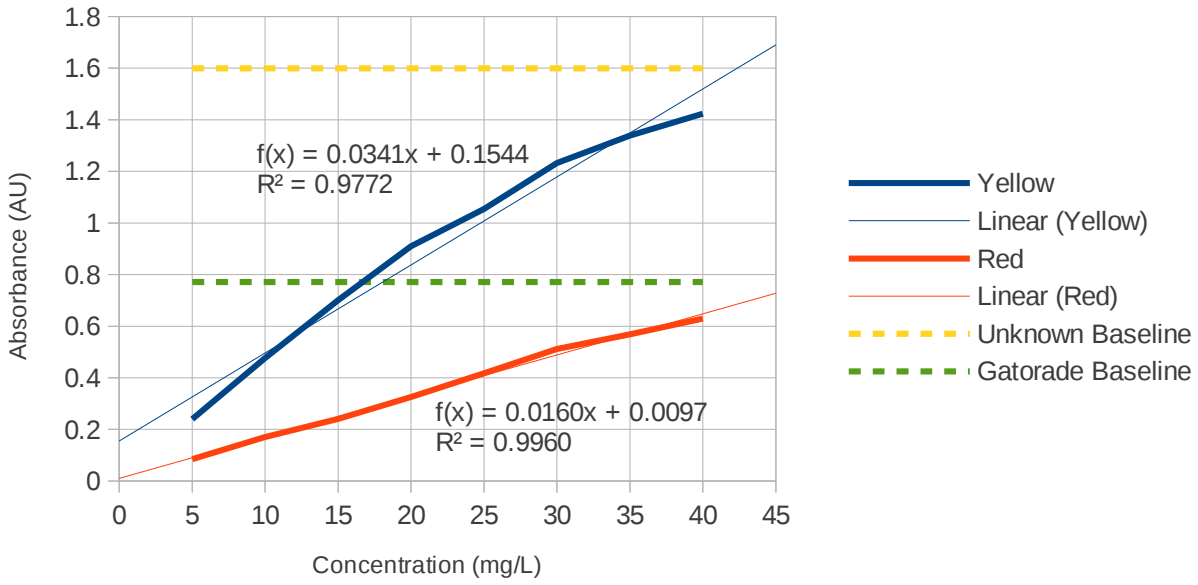

Figure 5: Absorbance vs. Concentration for All Samples at 423.8 nm

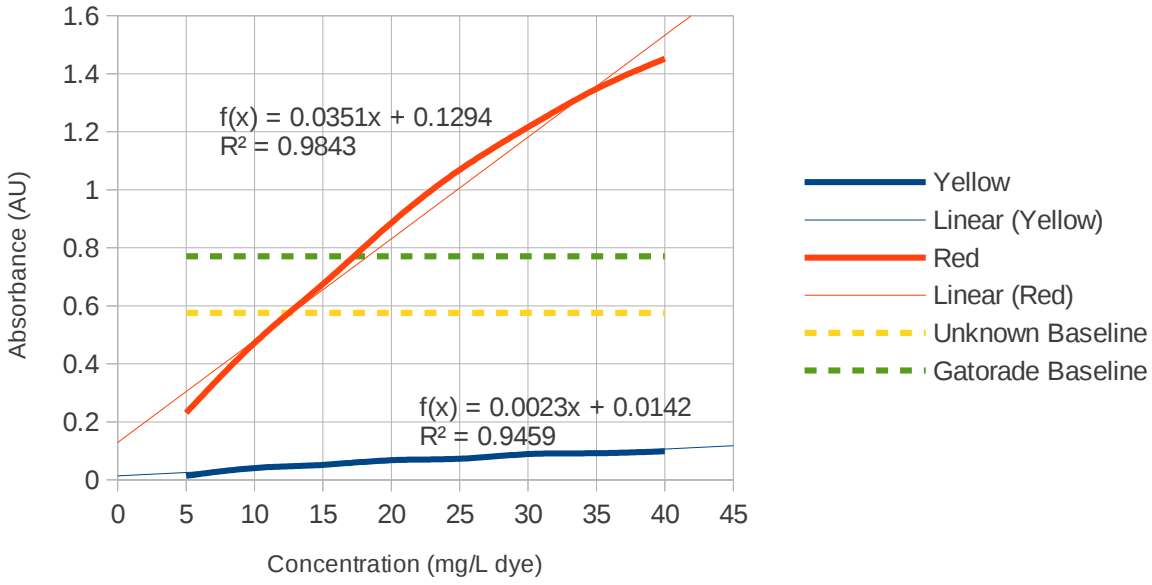

Figure 6: Absorbance vs. Concentration for All Samples at 501.3 nm