Alvarium Engineering

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MEMORANDUM

DATE: **Jan 26, 2025**
TO: **Engineering Training Supervisors: Butterfield, Firth, Mohanty**
FROM: **Group 3D2: Simone Burdick, Elijah England, Aidan Mulligan**
SUBJECT: **Evaluation of Our Low-Cost Photometer**

Summary:
The task at hand for week 3 of 1705 was to finish making our Photometer and then use it to collect data- which would test if our build worked. We 3D printed a box in which we placed two breadboards. We also added stilts to allow for our tubing to go all the way through during the Flow Cell portion of our lab. One breadboard was wired to be a photometer, and the other was our light. We did have to seal some holes that could let in excess light, as well as chip some pieces away to make our 3D printed parts fit together better. Since we had met earlier that week to make sure that everything was fitting together well, we were able to start calibration immediately. When we calibrated our photometer, we used the water sample, the black sample, along with A1, A7, B4, and C3. After we collected that data, we went to the Reaction Station and collected data from a mixture of 1mL of NaOH and 2.5mL of Fuchsin. When it was time for the Flow Cell Station, we added our stilts to the photometer build, to allow for room for the tube to go through. All experiments were run successfully, though we did have to re-do the first two due to user error. We started and nearly completed our write-up on the day of our lab. By doing this, we were able to find values such as ε, k, and α. Our Molar Extinction Coefficient, ε=0.00579; our k=0.0218; and our stoichiometric coefficient on fuchsin, α=1.

Apparatus and procedure:

Apparatus:

The spectrophotometer was built using electronic parts sourced from the University of Utah MIL lab, which is operated by the University of Utah Department of Chemical Engineering. To source the required materials for this project, please see Figure 2.

The main idea behind the design was to move the breadboard inside of the shell, as opposed to having the circuit on the outside. One benefit of this was that the spectrophotometer remained as a single enclosed system, and is just one object. However, this design required a larger housing and more filament to print. See Figure 1A.

Another difference with this design was the choice to use two seeeduino microcontrollers as opposed to just one. The two microcontrollers was a result of a previous design in CH EN 1703, where one of the microcontrollers was measuring temperature. It was easier to have two microcontrollers measuring temperature and light absorbance separately. Although not measuring temperature, this design choice was kept in the new version. See Figure 1B.

Procedure:

The photometer was calibrated by the construction of a calibration curve, based on Beer's Law. This calibration curve was performed on a single continuous run of the spectrophotometer, and different pre-prepared samples of known fuchsin concentration were sequentially placed into the detection spot. They were tested from least to most concentrated in the following order: $0M, 7.14 * 10^{-7}M, 5.71 * 10^{-6}M, 2.86 * 10^{-5}M, 1.0 * 10^{-4}M$. Finally, the maximum absorbance solution was tested. These different solutions were chosen due to their wide range of concentrations, spanning multiple orders of magnitude. This would give the photometer a very wide range.

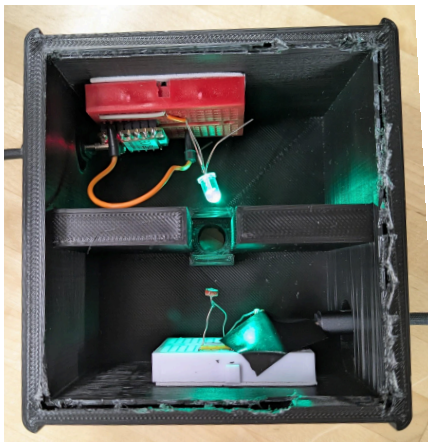
During the batch reaction, 2.5 mL of diluted stock NaOH solution was added to 1.0 mL of stock fuchsin solution in a cuvette. The cuvette was then instantly put into the spectrophotometer, to measure the reaction as quickly as possible. As the reaction progressed, the mixed solution became more and more translucent, which was measured by the photoresistor.

Functionality of the flow cell was validated by putting piping through a hole in the top and bottom of the photometer, and then flowing liquid from the bottom to the top of the photometer. This line passed in front of the photoresistor, allowing for an absorbance reading. First, the fuchsin solution was put through the line. After about 30 seconds, the flow was switched to water, which should be less absorbent. Finally, the flow was changed back to the fuchsin solution at the end of the run, confirming that the flow cell worked.

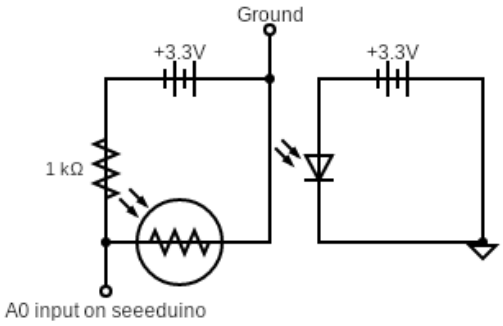
Please see the attached spreadsheet, which is also being shared. This spreadsheet contains all calculations for the calibration curve, batch reaction, and the flow cell. A link is attached: https://docs.google.com/spreadsheets/d/1yBI1K0RKmnJHOXLCU_z2M2MTtsjtvq3JvZScfFSWzr0/edit?usp=sharing

Figure 1: Photometer Design

(A)



(B)



(C)

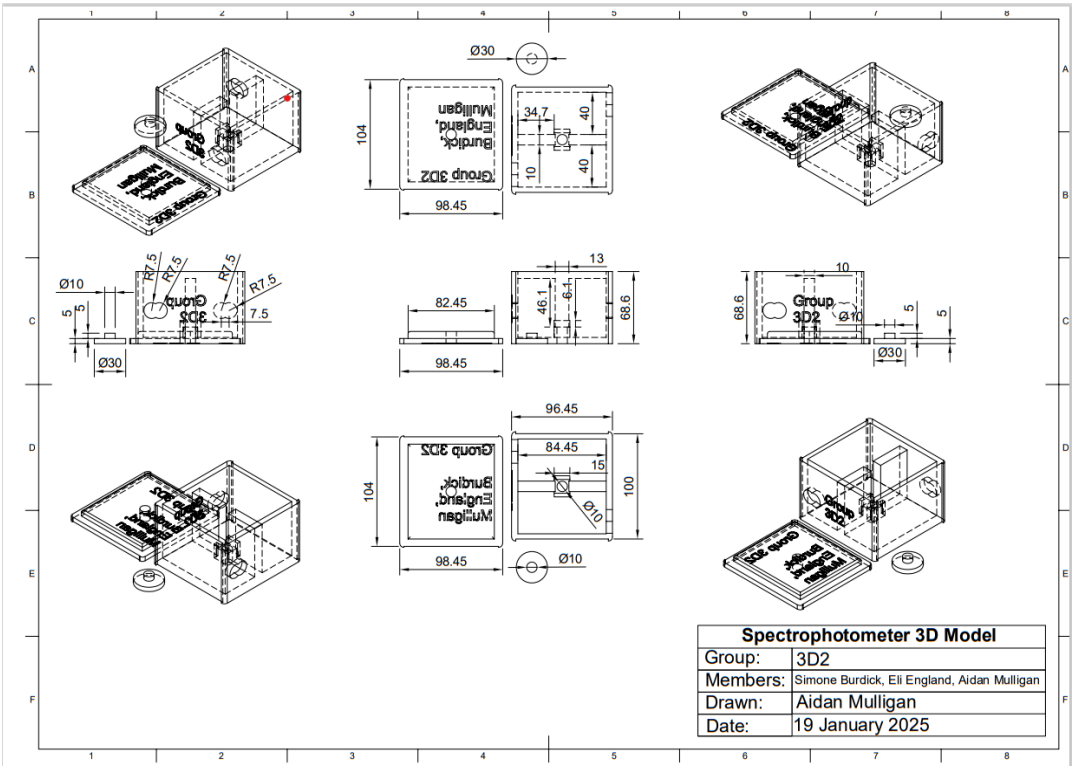


Figure 1: The figure above depicts the design of the spectrophotometer used.

(A) This picture of the photometer shows the LED (top). The LED shines light through a hole (center) that holds the sample in a cuvette or pipe. After passing through the sample, light hits the photoresistor (bottom), which is used to determine sample concentration.

(B) This is a diagram of the circuits that were used. The left circuit pin is connected to a seeeduino

(C) This is a diagram showing the dimensions of the 3D model that was printed for the photometer. Important things to note are the walls separating the resistor and LED, location of holes for power cords, covering lid, and round hole plug.

Figure 2: Table of Components and Cost

Component	Source	Unit Cost (USD per unit)	Quantity	Cost All Units (USD)
Seeed Studio XIAO Microcontroller	amazon.com microcontroller	$9.90 ea	2 units	$18.80
Small breadboard	amazon.com breadboard	$1.00 ea	2 units	$2.00
Cuvette	amazon.com cuvettes	$0.44 ea	1 unit	$0.44
Cuvette lid	amazon.com cuvette lid	$1.85 ea	1 unit	$1.85
Jumper wire kit	amazon.com wire kit	$6.49 ea	1 unit	$6.49
GL5506(A) Photoresistor	amazon.com GL5506(A) Photoresistor	$0.30 ea	1 unit	$0.30
0.6cm diameter tubing	amazon.com 0.6cm diameter tubing	$1.10 per meter	56cm	$0.62
1000 ohm resistor	amazon.com 1kohm resistor	$0.12 per resistor	1 unit	$0.12
Black PLA filament	amazon.com black PLA filament	$17.99 per kg	~170g	$3.06
RGB LED Diode	amazon.com RGB LED Diode	$0.09 per LED diode	1 unit	$0.09
				TOTAL COST: $33.77

Results:

The first results that were attained were from the Beer's Law calibration curve (Figures 4A and 4B). This curve, as with all this group's data, was taken using a green light (550 to 580 nm). The calibration data provided strong initial data that this team's spectrophotometer was performing successfully. In the Abs/Conc chart, for instance, the R^2 value was 0.99 (Figure 4B). This indicates that the calibration curve data was extremely well fitted to the line of best fit, and that there was little noise. The same was also true for voltage plotted against concentration, though this wasn't quite as strong of a linear fit with an R^2 value of 0.938 (Figure 4A). The likely reason for this difference is that voltage may not have a completely linear relationship with concentration. The range of the photometer was quite broad. At 0% transmittance, the average voltage reading was 0.457V. Similarly, at 100% transmittance the average voltage reading was 1.944V. As neither of these voltages maxed out the microcontroller, they were evidence of a wide reading range. The molar extinction coefficient (ε) was found to be $\varepsilon = 5.793 * 10^{3} M^{-1} cm^{-1}$. This molar extinction coefficient was significantly different from the literature value at 539, which is 8.6e6 1/(M cm). The difference is about three orders of magnitude. It is entirely possible that the difference was a result of the wavelengths selected for the calibration. We performed our calibration in green light, which has a wavelength of between 550 and 580 nm. This range is 11 to 41 nm different from the literature value, which may possibly result in the difference.

Figure 3: Calibration Curves

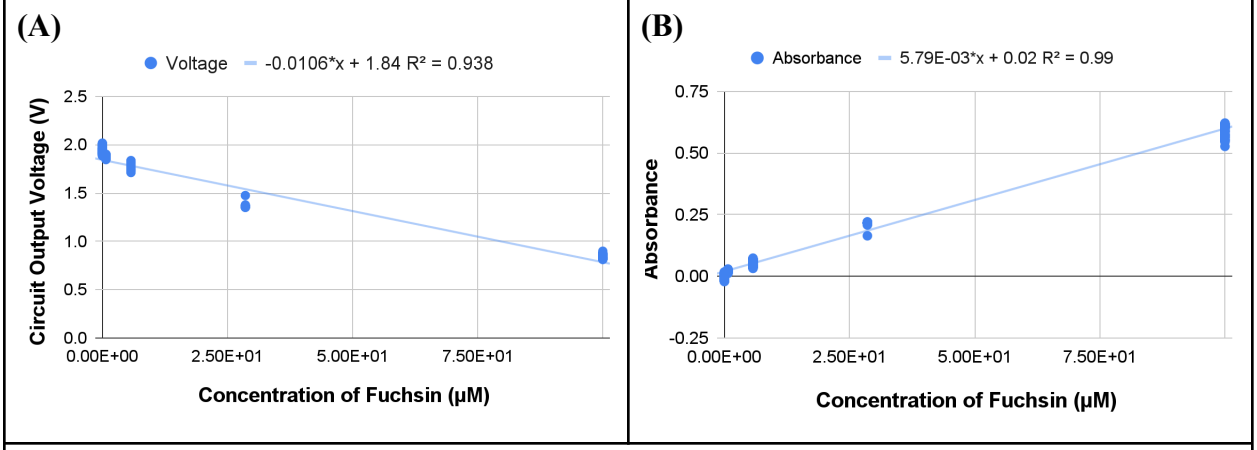


Figure 4: The molar extinction coefficient (ε) was determined experimentally by constructing a calibration curve. This calibration curve was based off of Beer's Law, using green light (550-580 nm). At 550-580 nm, the value of the molar extinction coefficient (ε) is: $\varepsilon = 5.793 * 10^{3} M^{-1} cm^{-1}$.

(A) This chart shows the output voltage of the circuit (V) as a function of fuchsin concentration (µM). At this wavelength range, the average voltage at 0% transmittance was 0.457V, and the average voltage at 100% transmittance was 1.944V.

(B) This is the calibration curve for determining the expected absorbance (unitless) as a function of fuchsin concentration in µM. The value of ε was $\varepsilon = 5.793 * 10^{3} M^{-1} cm^{-1}$.

In Figures 4A and 4B, a reaction between fuchsin and NaOH was plotted. By recording the absorbance from fuchsin over time, the order of this reaction could be determined. In Figure 4A, the concentration was plotted against time, revealing an R^2 value of 0.905. In Figure 4B, the natural log of concentration was plotted against time, revealing an R^2 value of 0.956. The R^2 value from Figure 4B is thus significantly higher than the R^2 attained in Figure 4A. Because the natural log of concentration was a better fit against time than just concentration, this indicates that the reaction that occurred was first order. With this knowledge and the trendline from 4B, our estimation of the rate constant is 0.0218 (the - slope of the trendline) and the stoichiometric constant for Fuchsin would be 1.

Figure 4: Reaction Data

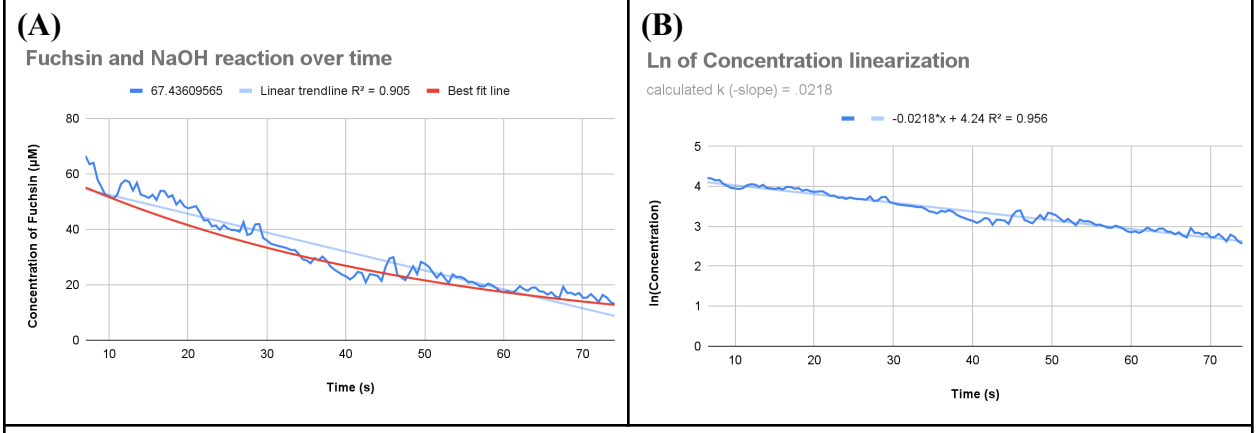


Figure 4: This was the reaction data for a reaction between 2.5 mL of fuchsin solution and 1.o mL of NaOH solution. The NaOH was in great excess.

(A) This graph shows how the concentration of Fuchsin decreases throughout the extent of the reaction, where concentration related to the following equation: $[C](t) = [C]oe^{(-kt)}$.

(B) This graph is the linearized concentration of Fuchsin over time, by taking the natural log of it. It also displays a trend line or best fit line along with an R^2 value.

The final results were attained from the flow cell operation (Figure 5). The flow cell was confirmed to work, as there was a highly significant difference between the clear and dyed flow (Figure 5). The average value of the clear flow achieved a voltage of 0.0789v, with a standard deviation of just 0.000970v. On the other hand, the dyed flow achieved a voltage of 0.0505v, with a standard deviation of 0.00100v. Because the standard deviations are extremely far away from each other and don't overlap, this result can be called significant. However, one thing to note is that the voltages read in the flow cell operation were quite different from those in the previous calibration and reaction tests. They demonstrate that the flow cell works, but should not be used to determine concentration in the flow cell.

Avg (clear): 0.0789 Std Dev (clear): 0.000970
Avg (dyed): 0.0505 Std Dev (dyed): 0.00100

Figure 5: Flow Cell

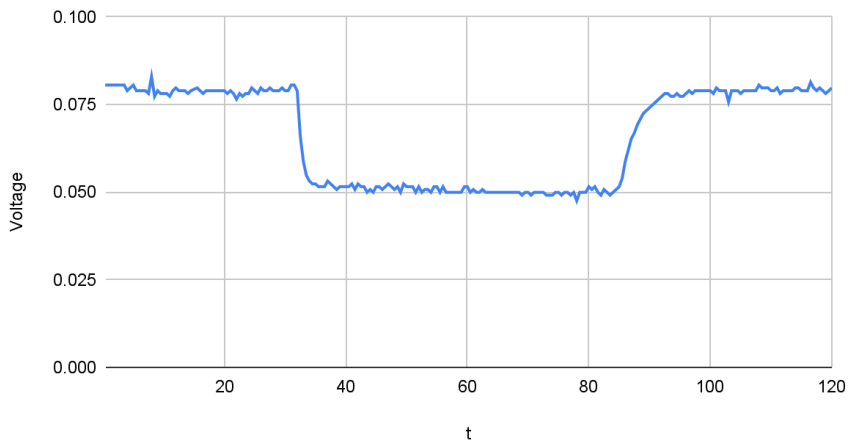


Figure 5: This is the graph for the flow cell data and the change in voltage. For the first ~30 seconds the dark fuchsin solution was flowing through the cell. Then, between ~30 and ~85 seconds, the clear solution went through the cell. Voltage was lower with the clear solution. Finally, the fuchsin stream was turned back on, and voltage went back up.

Conclusions:

Our spectrophotometer worked very well. The sensor and the light being separate was optimal as it allowed a clear and large separation wherein the tubing and cuvette could very easily fit in. There was also a barrier on either side of the cuvette, allowing the light to go to the sensor only through the cuvette rather than much excess light meeting the sensor. An adjustment we could have made to allow the light visibility to best function in that way through the tube would be to add 3D printed inserts on either side of the tube to block that same excess light that was blocked out with the cuvette. The distance between the barriers and the tubing was less, but our print ended up being too narrow for our original tube, so we had to downsize. Leaving extra room for error such as that, and then just using tape to better close the holes would be a good idea for the future. Making the stilts a little taller to factor in for the height of the bent tube would be more optimal as well, since we had to tape the spectrophotometer build to its stilts to keep the two together without the tube knocking it over.

We were tasked to comment on the photometer designs of other teams as well. The team with the highest device precision with the cuvette with the least noise was Section 2's group G from Thursday. It had the lowest standard deviation in its results. The section with the highest sensitivity was Section 2's group E from Thursday. Finally, the section with the best flow cell performance was Section 2's group B since it has the largest change in voltage while still maintaining low noise.