

## **Noise and Detection Limits (NDL)**

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CHEM438 - 021L

*I submit this laboratory report as an original document. I assert that all ideas and discussion of data contained herein are my own work, unless otherwise referenced.*

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## ABSTRACT

To determine the accuracy limitation of Beer's Law at high absorbances, 5 sample solutions were prepared from diluting 1, 2, 3, 6, and 13 mL of 0.0011 M quinine sulfate stock solution in the 25-mL volumetric flask with 0.05 M H<sub>2</sub>SO<sub>4</sub> diluent. The calibration curve obtained showed a limiting absorbance of 3.271 and 3.273 through the graphical and calculation methods respectively, which were in agreement within the 0.002 absorbance unit. The ratio of  $\frac{P_{background}}{P_0}$  was found to be 0.00053. The limit of detection (LOD) at 1, 7, and 15-s integration times with 64, 448, and 960-s acquisition times were  $1.02 \times 10^{-4}$ ,  $6.36 \times 10^{-5}$ , and  $3.01 \times 10^{-4}$  in absorbance units respectively. It was observed that a longer integration and acquisition time can improve the LOD, in which the LOD in molar concentration unit for the respective time was 10.51, 6.53, and 30.86 nM.

## INTRODUCTION

Beer-Lambert's Law predicted that the concentration of solution is directly proportional to its absorbance data. However, there are a few limitations to Beer's Law. One of them includes the accuracy limitation at high absorbances. At a high absorbance region, Beer's Law linear relationship no longer holds. The power of the detector signal of the background measured is greater than the power of the signal of the sample solution itself. The instrument's signal is dominated by noise, which does not provide accurate data measured.

The second limitation to Beer's Law is the presence of noise affecting the detection limit. The absorbance decreases and the light power recorded by the instrument has a value that is approaching the light power of a blank sample. Fluctuations in the intensity of the light source is causing the noise in the blank sample. This fluctuation in reality behaves as random noises or drifts with time.

## PROCEDURE

### *Part I: Determination of the absorbance upper limit<sup>1</sup>*

A stock solution of quinine sulfate,  $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$  was prepared by adding 0.0427 g of the salt into a 50-mL volumetric flask and diluted up to the neck of the flask with 0.05 M  $H_2SO_4$  solution. 5 sample solutions were made by pipetting 5 different volumes (1, 2, 3, 6, and 13 mL) of the stock solution using the volumetric pipettes into 5 separate 25-mL volumetric flasks and diluted with the same diluent as the stock's.

Agilent model 8452 UV-Vis absorbance spectrometer was used to collect the absorbance data at the wavelength of 340 nm. The absorbance of the blank sample (the diluent) was measured first, followed by the 5 sample solutions.

### *Part II: Determination of the detection limit<sup>1</sup>*

Using the same instrument, absorbance at a wavelength of 340 nm with an integration time of 1 s (acquisition time of 64 s) was collected, by measuring the blank sample. The measurement was repeated twice, by changing the integration time to 7 and 15 s, with the acquisition time of 448 and 960 s respectively. The recorded absorbances were compared based on their detection limit.

## RESULTS AND DISCUSSION

### Part I: Determination of the absorbance upper limit

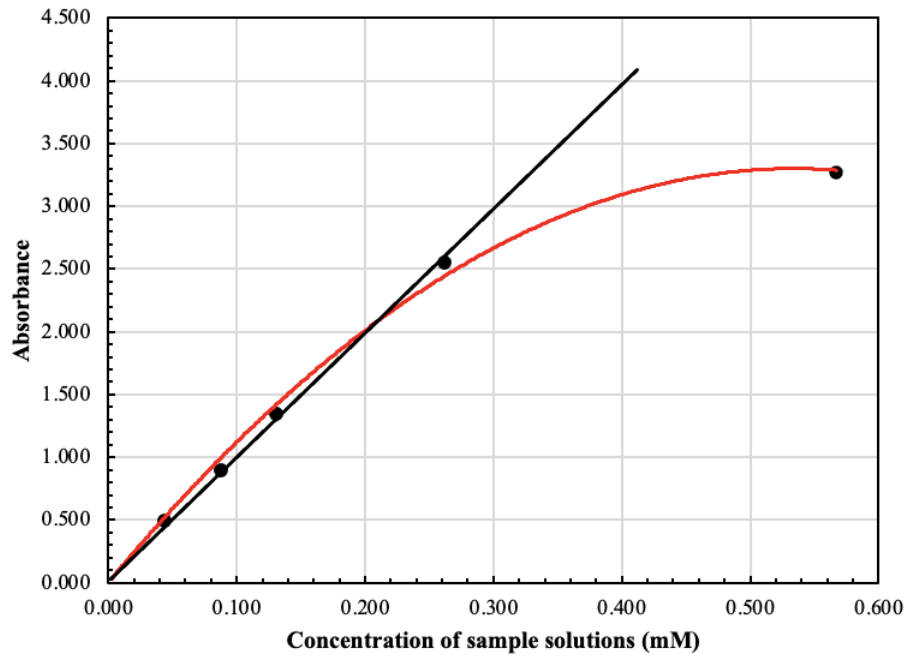
**Table 1.** Absorbance data collected for 5 sample solutions.

Sample	<sup>a</sup> V <sub>Added</sub> (mL)	<sup>b</sup> A	<sup>c</sup> Concentration (mM)
1	1	0.490	0.044
2	2	0.897	0.087
3	3	1.342	0.131
4	6	2.556	0.262
5	13	3.271	0.567

<sup>a</sup> Variation in volumes of 0.0011 M stock solution added into each flask

<sup>b</sup> Absorbance at  $\lambda = 340$  nm

<sup>c</sup>  $A = \epsilon bc$ ,  $\epsilon = 9750 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ,  $b = 1 \text{ cm}$ , where  $c$ : concentration (M)



**Figure 1.** Plot of absorbance against sample solutions' concentration collected at  $\lambda = 340$  nm.

**Table 1** summarized the absorbance data collected at wavelength,  $\lambda$  of 340 nm and the final concentration of the sample solutions. These data were plotted as shown in **Figure 1**. The black line represented the ideal case with zero background noise, while the red curve demonstrated the limitation of Beer's law at high absorbance due to the presence of background noise in the data collected. The red curve was obtained by fitting **Eq. 1** to the data points plotted.

$$A_{\text{apparent}} \simeq -\log \left[ 10^{-\epsilon bc} + \frac{P_{\text{background}}}{P_0} \right] \quad \text{Eq. 1}$$

It could be measured graphically the limiting absorbance of the data set. Based on **Figure 1**, the limiting absorbance would be approximately 3.271, which was the same as the absorbance data of the fifth sample solution. By rearranging **Eq. 1**, **Eq. 2** was obtained and used to determine the value of  $\frac{P_{background}}{P_0}$ .

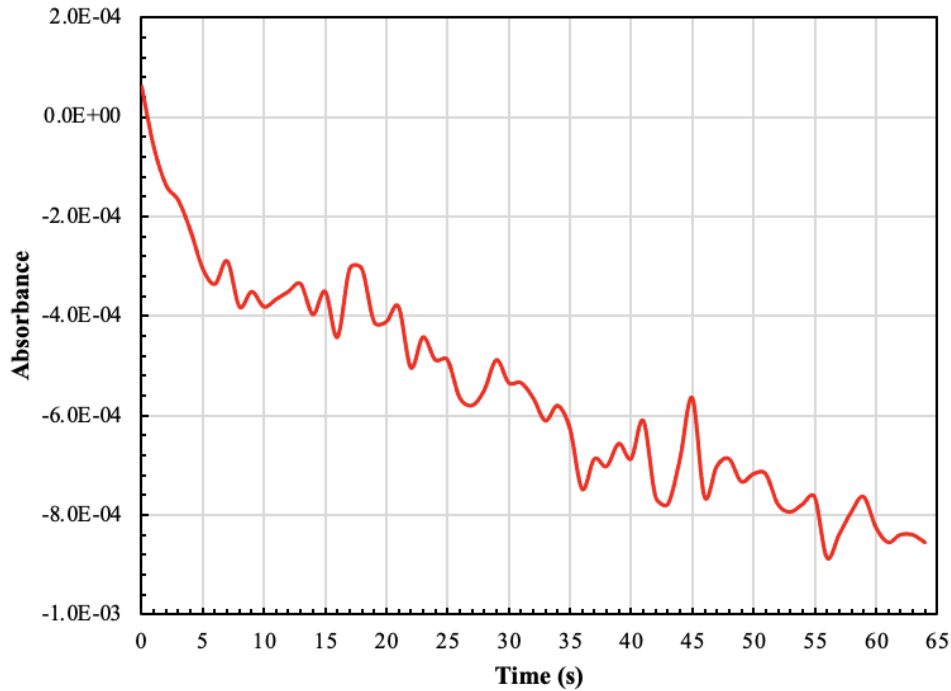
$$\frac{P_{background}}{P_0} = 10^{-A_{apparent}} - 10^{-\epsilon bc} \quad \text{Eq. 2}$$

The deviation of absorbance data from linearity was due to the domination of background light power,  $P_{background}$ , rather than the detector signal of the sample itself,  $P$ . The signal-to-background ratio was less than one. The presence of background signals caused the value in  $P$  to be insignificant. This relationship was used to rederive **Eq. 1** to form **Eq. 3**, to determine the exact value of limiting absorbance.

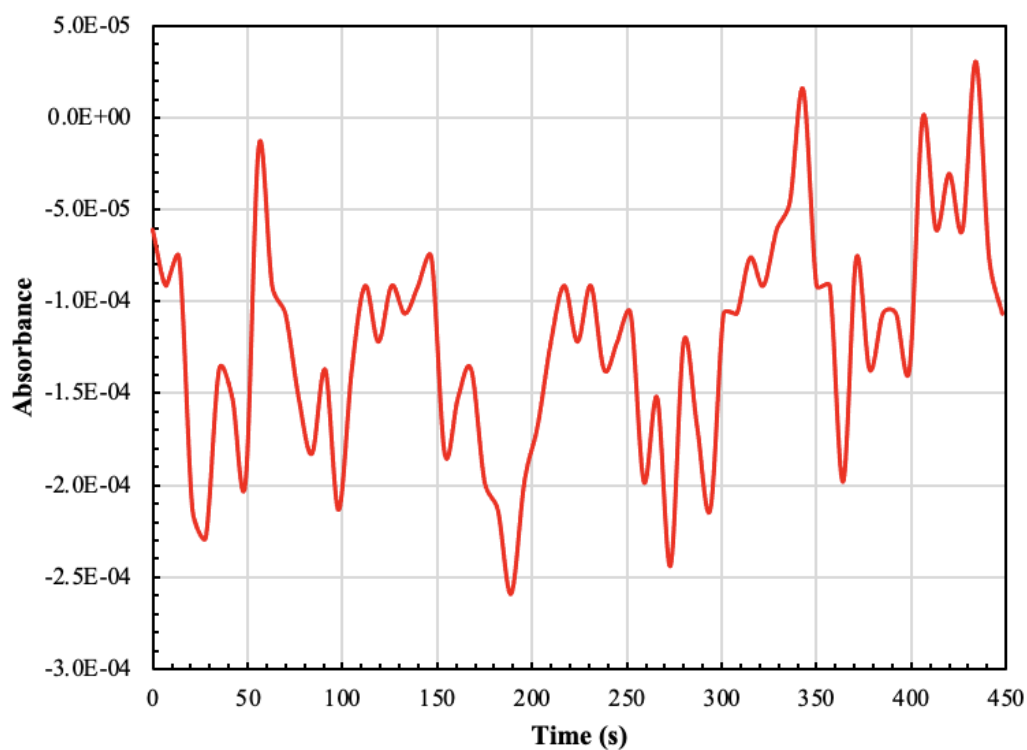
$$\lim_{P \rightarrow 0} (A_{apparent}) = -\log \left( \frac{P_{background}}{P_0} \right) \quad \text{Eq. 3}$$

It was found that the value of  $\frac{P_{background}}{P_0}$  was 0.00053, which yielded a limiting absorbance of 3.273. The difference in estimated and calculated limiting absorbance values was only 0.002, hence the limiting absorbance are in agreement.

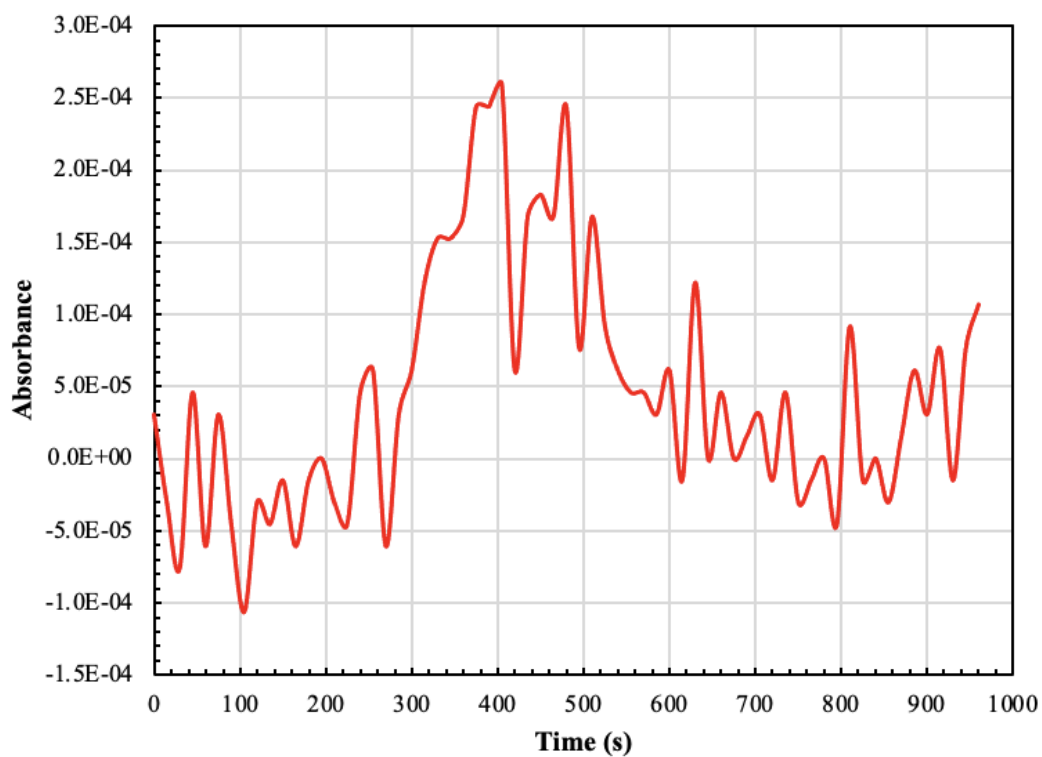
## ***Part II: Determination of the detection limit***



**Figure 2.** Absorbance data at 1-s integration time, for a duration of 64 s.



**Figure 3.** Absorbance data at 7-s integration time, for a duration of 448 s.



**Figure 4.** Absorbance data at 15-s integration time, for a duration of 960 s.

**Table 2.** Limit of Detection for three integration times.

Integration Time (s)	<sup>a</sup> $\bar{A}_{blank}$	<sup>b</sup> $\sigma_{blank}$	<sup>c</sup> LOD (au)	<sup>d</sup> LOD (nM)
1	$-5.54 \times 10^{-4}$	$2.19 \times 10^{-4}$	$1.02 \times 10^{-4}$	10.51
7	$-1.22 \times 10^{-4}$	$6.17 \times 10^{-5}$	$6.36 \times 10^{-5}$	6.53
15	$4.55 \times 10^{-5}$	$8.51 \times 10^{-5}$	$3.01 \times 10^{-4}$	30.86

<sup>a</sup> Mean of the blank absorbance over the entire period

<sup>b</sup> Standard deviation of the blank absorbance over the entire period

<sup>c</sup> Limit of detection in absorbance unit

<sup>d</sup> Limit of detection in molar concentration units of quinine

**Figure 2 - 4** show the variation in blank's signal for the integration time of 1, 7, and 15 s respectively. The mean and standard deviation of the blank's absorbance for each data set were computed and tabulated in **Table 2**. The mean was expected to be very small, as the absorbance of a blank (0.05 M H<sub>2</sub>SO<sub>4</sub>) should give out absorbance of near to 0. The standard deviation for 1-s integration was expected to be greater than the other two integration times. This was related to the length of the acquisition time applied on the instrument for each integration-time data set. If the instrument was set up to have a shorter acquisition time, the absorbance data was observed to be drifting more than what was expected from the longer acquisition times. The data were collected within a shorter time period that the absorbance could not vary much within 0. The absorbance for the 7 and 15-s integration time on the other hand behave as random noise. It could be seen that for the 448 and 960-s acquisition time for each case respectively, the absorbance fluctuate up and down randomly, and not drifting to one direction (as it was going down in **Figure 2**).

In other sense, looking at specifically **Figure 3** within 0 to about 25 s, the absorbance seemed to be drifting throughout the time span. However, the absorbance would behave as random noise over long periods of time, as the absorbance data fluctuated up and down after the 25-s point of time. The same observation could also be made from **Figure 4**.

The limit of detection (LOD) of the sample data set in both absorbance and concentration units could be determined based on the mean and standard deviation of the blank's absorbance. **Eq. 4** was used to determine the LOD in the absorbance unit, and Beer's law as provided in the footer c of **Table 1** to determine the LOD in the concentration unit.

$$LOD (au) = \bar{A}_{blank} + 3\sigma_{blank} \quad \text{Eq. 4}$$

Longer integration times helped improve the detection limit. More absorbance data could be collected and the averaging of noise will be more accurate and reliable. The key was to have a stable measurement of the detection limit and this could only be achieved after a long period of time<sup>2</sup>.



## CONCLUSIONS

Beer's Law has its limitations, which include the signal is limited in accuracy at high absorbance and the presence of noise in the instrument affects the detection limit. From sampling the quinine sulfate solution, the absorbance deviates from linearity at the high concentration region as expected. Besides that, the absorbance data of a longer integration time with longer acquisition time will behave as random noise, as opposed to that of shorter integration and acquisition time which is drifting with time. Longer integration time improves the detection limit as the signal behaves just as random noise.

## REFERENCES

<sup>1</sup> Cruz, F. *CHEM438 Instrumental Methods Laboratory - Noise and Detection Limits lab*; University of Delaware: Newark, Delaware, 2020; pp 1-9.

<sup>2</sup> Sensitivity, Background, Noise, and Calibration in Atomic Spectroscopy: Effects on Accuracy and Detection Limits.  
[https://resources.perkinelmer.com/lab-solutions/resources/docs/WHP\\_Atomic\\_Spectroscopy-Effects\\_on\\_Accuracy\\_and\\_Detection\\_Limits\\_013559\\_01.pdf](https://resources.perkinelmer.com/lab-solutions/resources/docs/WHP_Atomic_Spectroscopy-Effects_on_Accuracy_and_Detection_Limits_013559_01.pdf) (accessed Oct 1, 2021).

# APPENDICES

## Lab Notebook

Exp. No. <u>2</u>	Experiment/Subject <u>CHEM 438 (NDL)</u>	Date <u>9/20/21</u>	<b>09</b>
Name <u>Abdul Fayed</u>	Lab Partner <u>Giana Alepa</u>	Locker/Desk No.	Course & Section No. <u>021L</u>

### Pre-Lab Assignment

upload to canvas!

i. Upper limit of A  $\sim 3$  (or 2.5, a bit lower)

Wavelength = 290 or 330 nm. ( $\lambda =$  use 340 nm)

For  $\lambda = 290$  nm, wave number =  $34,482.76 \text{ cm}^{-1}$

"  $\lambda = 330$  nm, " =  $30,303.03 \text{ cm}^{-1}$

molar extinction coefficient  $[\epsilon] \text{ M}^{-1} \text{ cm}^{-1}$

For  $\lambda = 290$  nm,  $\epsilon \approx 2750 \text{ M}^{-1} \text{ cm}^{-1}$

"  $\lambda = 330$  nm,  $\epsilon \approx 7900 \text{ M}^{-1} \text{ cm}^{-1}$

$\lambda = 340$  nm?

Upper Limit Absorbance	3		2.5		
$\lambda$ (nm)	290	330	290	330	0.56
$\epsilon$ ( $\text{M}^{-1} \text{ cm}^{-1}$ )	2750	7900	2750	7900	340
b (cm)	1.00	1.00	1.00	1.00	9750
Max. Conc. (M)	1.091E-03	3.797E-04	9.091E-04	3.165E-04	1
Conc. 1 (M)	5.455E-04	1.899E-04	4.545E-04	1.582E-04	5.744E-5
Conc. 2 (M)	2.727E-04	9.494E-05	2.273E-04	7.911E-05	2.872E-5
Conc. 3 (M)	1.364E-04	4.747E-05	1.136E-04	3.956E-05	1.436E-5
Conc. 4 (M)	6.818E-05	2.373E-05	5.682E-05	1.978E-05	7.179E-6
Conc. 5 (M)	3.409E-05	1.187E-05	2.841E-05	9.889E-06	3.590E-6
Min. Lowest Conc (M)	4.364E-05	1.519E-05	3.636E-05	1.266E-05	1.795E-6

$\Rightarrow$  some possible concentrations of Quinine -

Conc. 1 =  $5.673 \times 10^{-4} \text{ M}$

Conc. 4 =  $8.727 \times 10^{-5} \text{ M}$

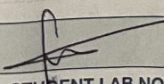
Conc. 2 =  $2.618 \times 10^{-4} \text{ M}$

Conc. 5 =  $4.364 \times 10^{-5} \text{ M}$

Conc. 3 =  $1.309 \times 10^{-4} \text{ M}$

$\Rightarrow$  Final concentration made!!

$\epsilon = 9750 \text{ M}^{-1} \text{ cm}^{-1}$   
Final  $\epsilon$  used!

Signature 	Date <u>9/20/21</u>	Witness/TA	Date
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Exp. No. <u>2</u>	Experiment/Subject <u>CHEM 438 (NDL)</u>	Date <u>9/20/2021</u>
Name <u>Abdul Payeal</u>	Lab Partner <u>Giana Alepa</u>	Locker/Desk No. <u>10</u>
		Course & Section No. <u>021L</u>

### PRE-LAB

2. No need to make new solutions. Just repeat the blank many times. ~~Can't sure why~~ To get the avg, std. dev. then LOD.

3.

Molar mass (g/mol)	782.96			
Stock soln. volume (mL)	50			
Stock soln. Conc (M)	1.091E-03	3.797E-04	9.091E-04	3.165E-04
Mass of salt for stock (g)	0.0427	0.0149	0.0356	0.0124
Total vol. after dilution (mL)	25	25	25	25
Vol. of stock soln. added (mL)				
1	12.500	12.500	12.500	12.500
2	6.250	6.250	6.250	6.250
3	3.125	3.125	3.125	3.125
4	1.563	1.563	1.563	1.563
5	0.781	0.781	0.781	0.781
Total volume added (mL)	24.219	24.219	24.219	24.219

Final Stock solution used!

13  
6  
3  
2  
1

Notes:

Consider which instrument to use, and jot it down!

For  $\rho_{\text{LOF}}$   
mass = 0.0022 g  
For stock of  
5.744 E-5 M

Final volume added

13, 6, 3, 2, 1 mL

in order of conc. page mentioned from prev.

⇒ Possible mass of guanine sulfate to be added.

4. If  $P_0$  is drifting, it will hurt the LOD, because it impacts sensitivity. Noise is from the fluctuations of lamp intensity, which are random on short time scales, but slowly drifting over long time scales. Recording absorbance over time and applying the slope of the drift to correct the measurements can detect the drift. Take measurement with a blank for a shorter amount of time. ~~to~~

Signature <u>[Signature]</u>	Date <u>9/20/21</u>	Witness/TA	Date
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Exp. No. 2	Experiment/Subject CHEM 438 (NOL)	Date 9/20/2021	11
Name Abdul Tayeem	Lab Partner Giana Alepa	Locker/Desk No.	Course & Section No. 021L

### Objective

Purpose of this experiment is to explore the limitations of Beer's Law, by using the UV-vis instrument to collect the data. Student will be exposed of experience in observing the behavior of noise in data and the estimation of detection limits.

All data saved in txt. file in a drive, nothing is recorded in this page.

### Introduction

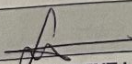
Beer's Law has 2 limitations:

a) Accuracy limit at high absorbance, and b) Detection limit from the effect of noise due to light source. For a), there is a maximum absorbance where the absorbance still follow Beer's Law, before it curves down and disobey linearity. Signal due to background overpower the signal due to sample, which creates this inaccuracy at high absorbance. For b), added random noise fluctuates the data at both the high and low-concentration region. This will affect the detection limit of the sample and gives inaccurate data.

### Procedure

#### PART 1

1. Prepare 5 solutions from the concentration table from pre-lab.
2. Dilute the solution with 0.05 M H<sub>2</sub>SO<sub>4</sub>.
3. Record which instrument is used for each dilution.

Signature 	Date 9/20/21	Witness/TA	Date
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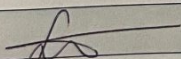
Exp. No. 2	Experiment/Subject CHEM 485 (NDL)	Date 9/20/2021	12
Name Abdul Foyeed	Lab Partner Giana Alepa	Locker/Desk No.	Course & Section No. 021L

## PART 2

1. Measure absorbance and record the data on a USB stick. TA will guide with the instrument.
2. In the instrument settings, use a cycle time equal to integration time.
3. Study the noise on the blank. Use "kinetics mode" in the software. Use only blank!
4. Choose  $\lambda = 340\text{nm}$ , integration of 1s.
5. Record blank measurement, then record signal for the same blank sample for 64s.
6. Save the data, using last name as file name.
7. Record a blank measurement for integration time of 7s, but acquire for 448s. (will give same ~~data~~ points)
8. Repeat step 7 using integration time of 15s, but with a 960-s acquisition time.
9. Dispose of all chemicals properly.

## Conclusions

Absorbances are collected at 340nm for all 5 concentrations. The data are saved in a text file to be analyzed in the final report. Same goes to the blank measurement collected at 3 different integration times.

Signature 	Date 9/20/2021	Witness/TA	Date
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