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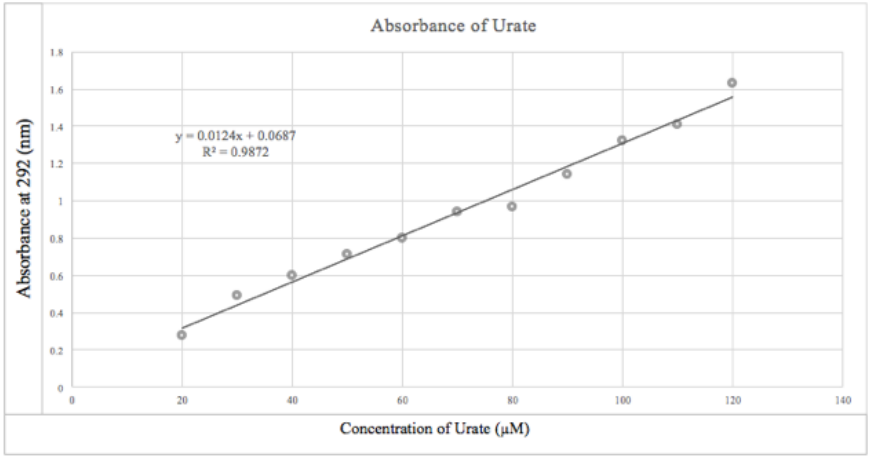
Experiment 1: Determination of the Molar Absorptivity of Urate

The goal of this experiment is to calculate the molar absorptivity of Urate at 293 nm. The uric acid must be dissolved in a buffer solution while maintaining a pH at or near 8.0 or else it will irreversibly react with oxygen to create either 4-hydroxyallantoin or parabanic acid[1].
In our experiment, we first created a TrisHCl buffer with a pH = 8.0. We added 3.63 grams of solid Tris to 250 mL of DI water. Next, we added 12.5 mL of 1.0 M HCl and filled the volume up to 300 mL. To 100 mL of this buffer solution we added 10 mg of solid uric acid to achieve a solution that is 600 µM urate. After this, solutions of varying concentration of urate were created with volumes of 10 mL each. The volumes and concentrations were as such:

Target [Urate], µM	120	110	100	90	80	70	60	50	40	30	20
V_{stock} (mL)	2.00	1.83	1.67	1.50	1.33	1.17	1.00	0.83	0.67	0.50	0.33
V_{tris} (mL)	8.00	8.17	8.33	8.50	8.67	8.83	9.00	9.17	9.33	9.50	9.67

The blanks were filled with just the TrisHCl buffer. When the spectrophotometer warmed up we ran the blank as a baseline correction. The first sample was run at 220-320 nm on very fast scanning speed and in absorbance mode. This was repeated for all eleven concentrations.



After our absorbances were read, we obtained a regression coefficient of 0.9872, a least square estimate of molar absorptivity of 12,382 $M^{-1}cm^{-1}$, a confidence interval of ± 1,946.6962

[1] Horinouchi, R., Yamamoto, Y., & Fujisawa, A. (2021). Increase of oxidation rate of uric acid by singlet oxygen at higher ph. *Journal of Clinical Biochemistry and Nutrition*. https://doi.org/10.3164/jcbn.20-101

$M^{-1}cm^{-1}$, and a relative error of 0.66% based on a literature value of (12,300 $M^{-1}cm^{-1}$)[2]. These results are fairly promising overall and suggest that our methods and rationale were satisfactory. One experimental design error that led to some of the error in our results was the sample size. While eleven samples is sufficient, the ease with which more samples could be run and the improvement in error justify running more samples. Somewhere in the range of 15 would significantly increase our confidence interval and presumably decrease our relative error. The majority of error; however, probably results from minor inaccuracies in the concentration of our Urate samples. While in theory our dilution holds, in practice, executing such a small change in volume multiple times over certainly led to human induced random error. There are two solutions to this proposed problem. Again, the first is to increase the number of samples, but the other is to start with a larger volume, so that small inaccuracies in the measuring of the initial mass of uric acid cause relatively less significant errors.

In the end, the objective of the lab was accomplished with error significantly lower than our worst-case-scenario of 15%. The molar absorptivity was obtained and a greater understanding of experimental design was attained. Further understanding of the spectrophotometer, the pH meter, and the micropipettes helped to elucidate the bounds with which our experiments are constrained within this lab.

Concentration of Urate (μM)	Absorbance at 292 nm
20	0.28
30	0.49
40	0.60
50	0.71
60	0.80
70	0.94
80	0.97
90	1.14
100	1.32
110	1.41
120	1.63

[2] Zhang, Chun, et al. “Impact of Large Aggregated Uricases and PEG Diol on Accelerated Blood Clearance of PEGylated Canine Uricase.” *PLOS ONE*, Public Library of Science, journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0039659.

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Calculations:

```
(*We want 300 mL of 0.1 M TrisHCl buffer with a pH of about 8.0*)
(*First we must determine the mass of Tris (s) to add*)
0.300 Liter * 0.1 mole / 1 Liter * 121.14 grams / 1 mole
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3.6342 grams
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(*In order to determine the amount of HCl we have two options.*)
(*A Use a pH meter while adding the HCl dropwise. Ideally we would stir and
 add slowly.*)
(*B Use the Henderson Hasselbalch equation to determine the concentration of
  conjugate base needed.
   pH=pK_a+log_10([A^-]/[HA]). Since pKa of uric acid is almost 8.0 we can just
   set [A^-] equal to [HA] so that the log term goes to 0. That is,
we want to add about 0.015 moles of 1 M HCl*)
0.015 mole / (1 mole/Liter)
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0.015 Liter
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(*We also want to know an appropriate concentration of urate to prepare. Thus,
we use the molar absorbtivity range provided in the prelab,      the given path length,
the given reliable range for absorbance in our spectrophotometer, and Beer's Law.*)
(* 2.0 ≥ A ≥ 0.005, 16000 1/(M*cm) ≥ ϵ ≥ 7000 1/(M*cm),b = 1 cm,
C=A/(b*ϵ)  *)
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2.857*10^-4 Mole/Liter ≥ C ≥ 3.125*10^-7 Mole/Liter;
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(*Since we want a Concentration of Uric acid to be between 2.857*10^-4 M and
 3.125*10^-7 we want to make a very dilute solution. We cannot do this by
 measuring out a very small weight due to the limited accuracy of our balances
 and we also do not want to make a very large volume of solution. So instead
 we performed a serial dilution on a 600 μM solution acquired by adding 10
 mg of uric acid to 100 mL of our buffer solution. We used this double diluted
 solution to prepare all of the following*)
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Target [Urate], μM	120	110	100	90	80	70	60	50	40	30	20
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```
(*95% confidence calculation*)
(* a±t(p,df)s_a *)
12 382 ± 2.26 * 861.37
12 382 ± 1946.6962
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(*relative error calculation*)
δ = | (V_A - V_E)/V_E | * 100
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