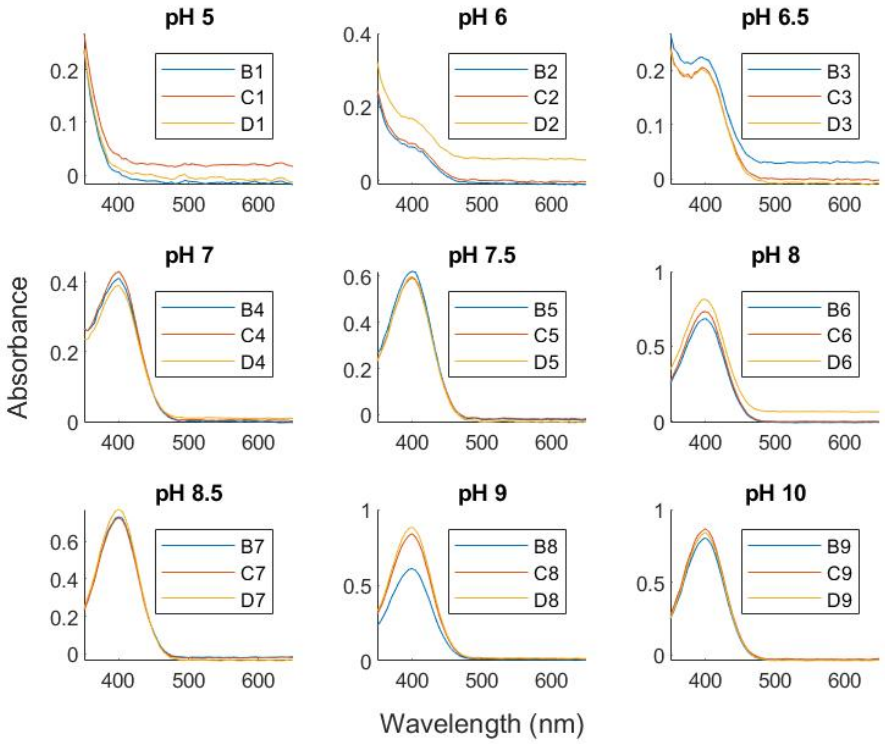
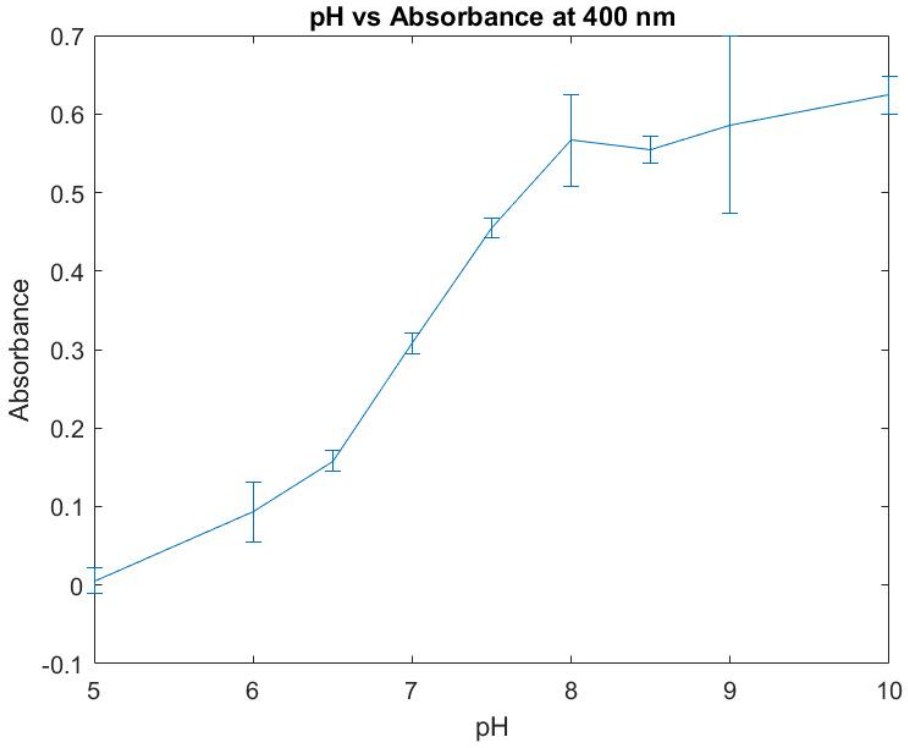
Part A

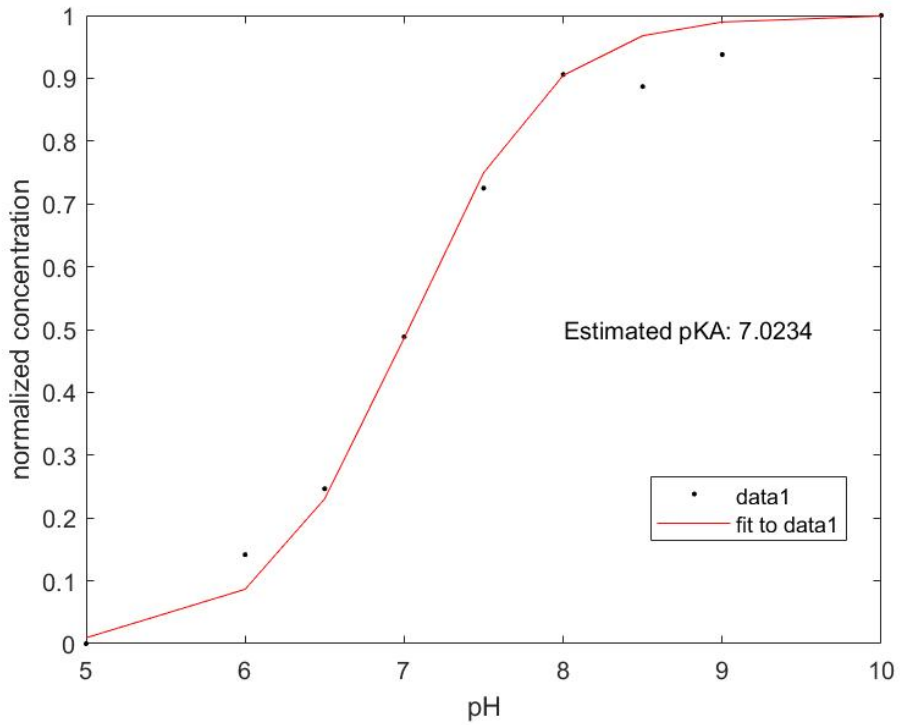
1. Plot absorbance vs. wavelength for PNP in all pH buffers. Be sure to subtract your blank (PNP free) trace from each sample trace. Which wavelengths show the most significant changes with pH? Which regions change the least? How does this relate to your visual observations?



2. Choose one wavelength that maximally distinguishes between the protonated and deprotonated forms of PNP. What is the advantage of choosing this wavelength? Make a plot of absorbance (at the chosen wavelength) vs. pH for all of your samples. Use your triplicate experiments to determine error bounds for each point and display using error bars.



3. Determine the pKa of PNP by fitting your data to a modified form of the Henderson_Hasselbalch Equation.

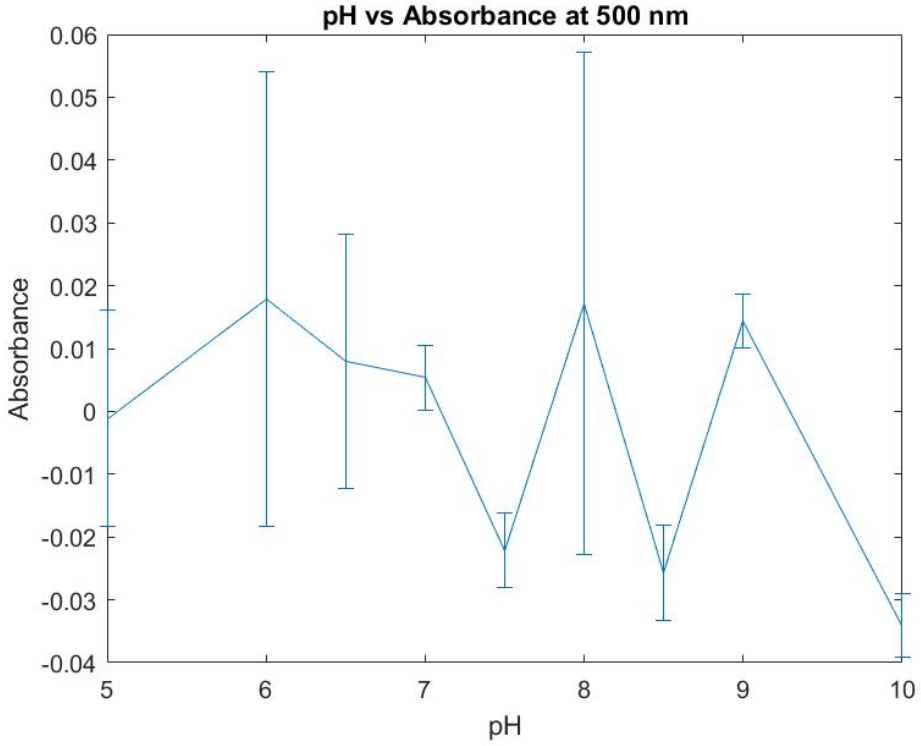


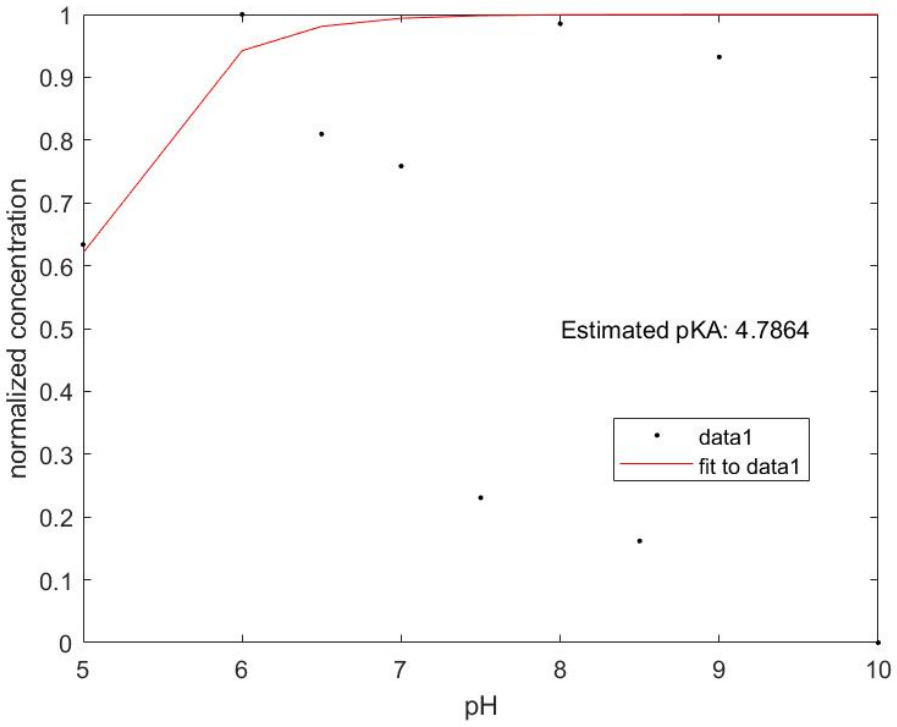
4. What is your estimated error on this value?

(TODO – How would I do this?)

5. Choose another wavelength that does not as optimally distinguish between protonated and deprotonated forms and again determine pKa with error bounds. How does this compare to the result of parts 3 and 4. Why are they different?

   At 500 nm, the pH/absorbance plot does not give any useful information. PNP does absorb much (if any) light at this wavelength for any pH, giving data that isn't very useful. This gives a garbage HH fit that isn't useful.





6. How do your measured values compare with existing published values?

   Published pKa = 7.15 (pubchem)

   Percent error = 100 * |(7.02 – 7.15) / 7.15| = *1.8% error*

Part B

1. Use your PNP absorbance vs. known pH to create a standard curve. Which wavelength did you choose and why?

   Question – What to do about normalization from part A?

   400 nm (max absorbance)

2. Use your standard curve to determine the pH of the unknown buffers and include error bounds. Are errors different for the different samples? Why or why not?

3. How well do your calculated values agree with the values you obtained using pH paper?

Part C

1. How many moles of PNP are in each well in the standard curve?

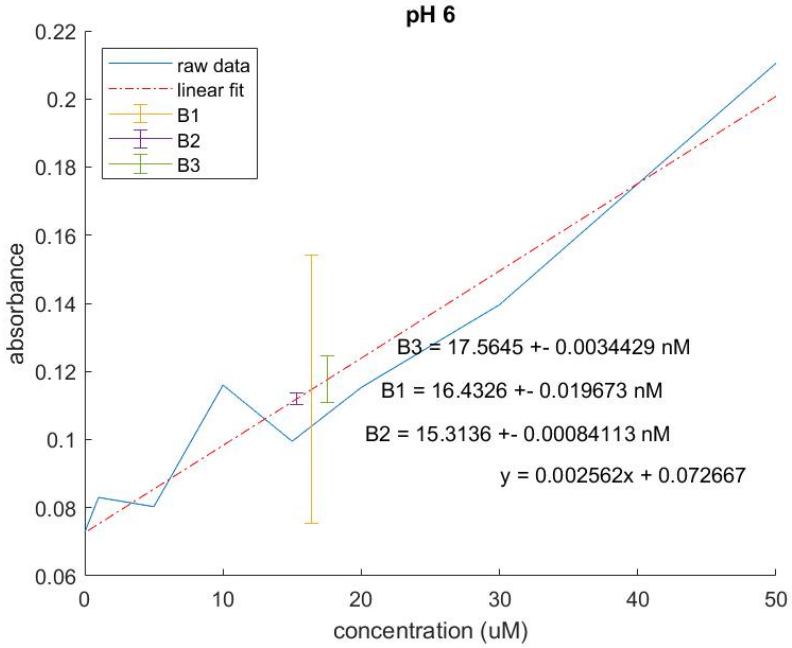
		1	2	3	4	5	6	7	8	9
Rows E/G	Conc (uM)	50	40	30	20	15	10	5	1	0

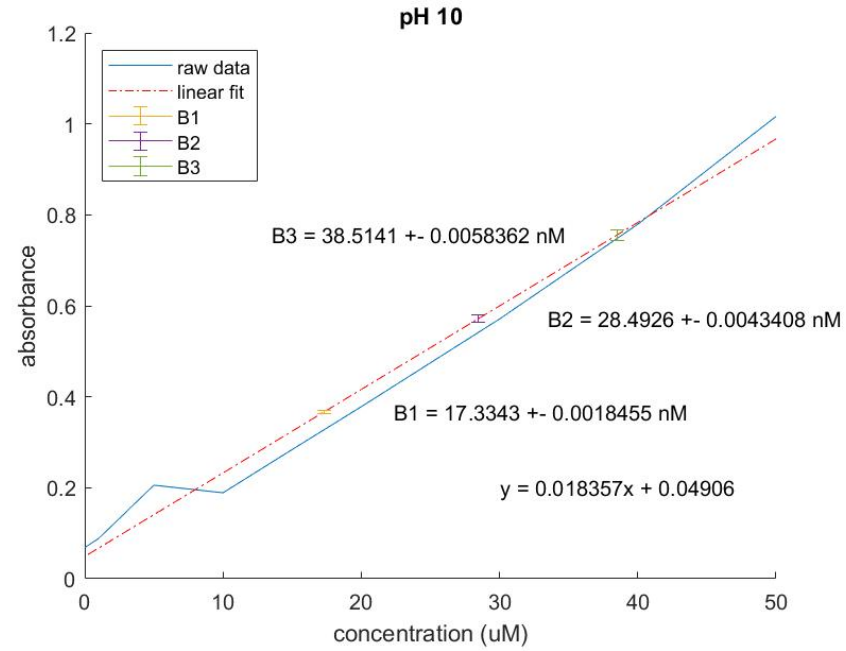
	mols	1E-8	8E-9	6E-9	4E-9	3E-9	2E-9	1E-9	2E-10	0

2. Which wavelength should you choose to create your standard curve?

   400 nm (max absorbance)

3. Plot absorbance vs. PNP concentration for your standard curve at the wavelength chosen above, including the blank (0 µM PNP).





4. Fit the standard curve to an appropriate function.

(see figure)

5. Use your fit to determine the concentration of your unknowns, with error bounds.

   (see figure, make sure to flip error bars)

6. How do your numbers vary in pH 10 vs. pH 6?

   pH 10 unknown estimates are more precise than pH6…