

Lab Experiment 2: Determining the pKa of o-nitrophenol.

Stock Solutions:

50 mM NaH₂PO₄ + 50 uM o-nitrophenol

50 mM Na₂HPO₄ + 50 uM o-nitrophenol

Determine the amount of each stock solution required to prepare the solutions in the table below:

pH	[o-nitrophenol]	Total Volume	Volume Acid	Volume Base
5	50 uM	3 mL		
5.5	50 uM	3 mL		
6	50 uM	3 mL		
6.5	50 uM	3 mL		
7	50 uM	3 mL		
7.5	50 uM	3 mL		
8	50 uM	3 mL		
8.5	50 uM	3 mL		
9	50 uM	3 mL		
9.5	50 uM	3 mL		

1. Mix each solution in a glass test tube.
2. Blank the spectrophotometer with dH₂O.
3. Read the absorbance of each solution at 410 nm.
4. Read the pH by transferring solutions to a glass test tube (you only need one).

Data Analysis

5. Plot A_{410 nm} vs. pH.
6. Fit the data to the following equation:

$$A_{410nm} = A_{deprotonated} - \left(\frac{A_{deprotonated} - A_{protonated}}{\left(10^{(pH-pKa)}\right) + 1} \right)$$

Fit Parameters:

A_{deprotonated} (the absorbance of the basic form)

A_{protonated} (the absorbance of the acidic form)

pKa

7. Use jackknife resampling to determine the error in the fit parameters and round appropriately.
8. Make a graph of A₄₁₀ vs. pH (see figure below).
9. Add your calculated Absorbance vs. pH to the graph.

10. Change the calculated data formatting on the graph to be a smoothed line and remove the data markers.
11. Add the fit pK_a with error to the graph
12. Polish your graph (see below). It needs to be scaled properly. Axes and axes labels need to be sized appropriately. Your plot should look like the figure below when you are finished.
13. How does your pK_a value compare to the accepted pK_a value for o-nitrophenol? Be sure to reference your value.

