Spectrophotometric Determination of pK_a of Phenol Red

This experiment uses instrumentation to accomplish quantitative analysis. *You will get far more experience in this during CH427 if you are a Chemistry or Biochemistry major*. In this experiment, the pKa of phenol red (an acid-base indicator) is determined through a combination of wet chemistry and spectrophotometric analysis. In lecture, we will learn that the advantage of a spectrophotometric titration over indicator-based titrations (and to some extent, potentiometric titrations) is that more of the titration curve contains information. In fact, you can determine an endpoint without ever sampling that point in the titration.

In this experiment we are doing a special type of titration to determine the acid dissociation constant K_a (rather than a concentration) – something you can also do with a potentiometric titration. We sample parts of the titration curve by constructing solutions of varying pH using buffers, rather than by sequentially adding titrant to a weak acid or base (which also forms buffers). Phenol red (phenolsulphonephthalein, $C_{19}H_{15}O_5S$) is a commonly used colorimetric pH indicator. It is a diprotic acid, H_2In, whose dissociations can be described by the following equations:

$$H_2In \rightarrow HIn^- + H^+ \qquad \text{Eqn-1}$$

$$HIn^- \rightarrow In^{2-} + H^+ \qquad \text{Eqn-2}$$

$$K_a = \frac{[H^+][In^{2-}]}{[HIn^-]} \qquad \text{Eqn-3}$$

It is the color change during the second dissociation that is usually used for indicator based titrations. HIn^- has a yellow color and In^{2-} has a red color. (This means their absorption spectra for the two chemical forms are different.) When both HIn^- and In^{2-} are present, the solution has an orange color. (Note 1) We take the log of Equation (3) and rearrange to give the linear form

$$pH = \log\frac{[In^{2-}]}{[HIn^-]} + pK_a \qquad \text{Eqn-4}$$

The ratio $\frac{[In^{2-}]}{[HIn^-]}$ is determined by spectrophotometry for several buffered solutions and their pH is also measured potentiometrically (using a pH meter). If pH is plotted against the log of this ratio (pH as the y-axis and the log ratio on the x-axis) the slope is close to 1 and the intercept is pK_a. {When $[In^{2-}] = [HIn^-]$, the log ratio is zero, making $pH = pK_a$}

PROCEDURE

If you want, you can pair up for this experiment. Prepare the solutions independently, and then come together for the absorbance measurements – this will give you two independent sets of data. As always, take turns doing the "hands-on" parts so that both partners will get the experience of using the instrument.

Overview

There are three major steps in the spectrophotometric determination of the ratios $[In^{2-}]/[HIn^{-}]$ for the buffered solutions and their use in the determination of pKa:

(1) Preparation of solutions with different pH values. An acidic solution of phenol red is prepared in which essentially all the indicator is in the HIn^{-} form. A basic solution is prepared in which essentially all the indicator is in the basic form In^{2-}. Buffered solutions (containing the same concentration of indicator) with different pH values are prepared, in which both In^{2-} and HIn^{-} are present. In all of the solutions the total concentration of indicator ($[In^{2-}] + [HIn^{-}]$) remains the same but their ratios vary with pH.

(2) Measurement of pH. The pH of the four buffered solutions will be measured using the benchtop pH meters according to the instructions provided in the pH Measurements section.

(3) Measurement of absorbances. The absorbance of all of the solutions will be measured at **550 nm** (the peak wavelength of the In^{2-} absorption band) according to the instructions provided in the Absorbance Measurements section and combined with the pH measurements in the calculation of pK_a

Calculation of pK_a

We start by defining the following absorbances at the selected wavelength 550 nm:
A_a = absorbance of HIn^{-} (*i.e.*, indicator prepared in the acidic solution, lowest in pH)
A_b = absorbance of In^{2-} (*i.e.*, indicator prepared in the basic solution, highest in pH)
A = absorbance of other mixtures (*i.e.*, indicator prepared in the buffered solutions)

The ratio of concentrations for the buffered solutions can be calculated from their absorbances as follows:

$$\frac{[In^{2-}]}{[HIn^{-}]} = \frac{(A - A_a)}{(A_b - A)} \qquad \text{Eqn-5}$$

We will use the acid and base solutions to find A_a and A_b, and then use those values with the absorbance values for each the four buffered solutions to generate the four concentration ratios. We will take the common log of each ratio and then plot the measured pH vs. the log of

the ratio from the buffered solutions. Finally we will plot the transformed data and use a linear regression to determine the "best fit" straight line. The y-intercept of this plot is the desired value, the pK_a of Phenol Red.

Solution Available

Phenol red solution has been prepared via the recipe below and is ready for you to use. Preparation: 0.050 g solid phenol red is dissolved in 50 mL of 95% ethanol and diluted to 250 mL with DI water.

Solutions to Prepare

1. Strong basic (0.1 M NaOH) solution (A_b): Add 2.00 mL of the phenol red solution to a 50 mL volumetric flask. (Note 2) Use a graduated cylinder to measure 5.0 mL of 1 M NaOH and add this to the 50 mL volumetric flask with the indicator, dilute to the mark, and mix thoroughly
2. 0.25 M KH_2PO_4: Weigh out 1.70 g of KH_2PO_4 and transfer into a 50mL volumetric flask dissolve in DI water, dilute to the mark, and mix thoroughly.
3. 0.25 M Na_2HPO_4: Weigh out 1.78 g of Na_2HPO_4 and transfer into a 50 mL volumetric flask, dissolve in DI water, dilute to the mark, and mix thoroughly. Na_2HPO_4 is really powdery, so if you find it hard to transfer the dry solid into small flask, you could dissolve them in 50mL beaker with small amount of DI water; then transfer the liquid into the 50-mL volumetric flask, dilute to the mark, and mix thoroughly.
4. Remaining solutions for the spectrophotometric measurements: To each of five 50 mL volumetric flasks add 2.00 mL of the phenol red solution. (Note 2) Then add the volumes of the two phosphate solutions according to the table below:

Table 1. Testing Samples preparation

Flask	KH_2PO_4 (mL)	Na_2HPO_4 (mL)	NaOH (mL)	Indicator (mL)	Composition
1	20	0	0	2	Acidic(A_a)
2	8	4	0	2	Buffered
3	5	5	0	2	Buffered
4	2	6	0	2	Buffered
5	0.8	6.4	0	2	Buffered

Dilute all 5 solutions to the mark with DI water and mix thoroughly.

Prelab Calculation: Measure the temperature of the solutions. The K_{a2} of phosphoric acid is 6.149×10^{-8} at 20 ºC and 6.303×10^{-8} at 25 ºC. Calculate the pK_{a2} at the measured temperature by interpolation. Then calculate the pH of flasks 2 through 5 using the value of pK_{a2} and the Henderson-Hasselbalch equation. *Note: because the phosphate solutions are much more concentrated than the indicator, you may neglect the influence of the latter in the calculation of pH. Otherwise this would be much harder (involving multiple equilibria).*

pH Measurements

Use the SympHony Benchtop pH meter to measure the pH of the four buffered solutions. In order for the tip of the pH probe to make full contact with the sample solution, we will use a dry dram bottle and add the sample you are going to measure until it is half-full. Rinse the pH probe with DI water between measurements to avoid carry-over. If the pH electrode has been stored dry, soak in storage solution or pH 7 buffer for 10 minutes before standardization to saturate the pH electrode surface.

Do not use the Kimwipe to wipe the tip of pH probe.

Procedure for calibrating pH meter

1. Turn the meter on, you should be able to see the standby screen as shown in Figure 1.
2. From the standby screen press the **Calibration** softkey.
3. Rinse the pH electrode with DI water then submerge it in the first pH standard buffer solution.
4. Press **Read** to measure the first calibration buffer solution. When the measurement stablizes, the instrument will request the next calibration buffer solution.
5. Rinse the probe with DI water and put the probe into the second calibration buffer solution.
6. Press **Read** to measure the second calibration buffer solution.
7. Repeat step 5 and 6 to measure the subsequent calibration points. *(3 points calibration for the benchtop pH meter)*
8. If the calibration is successful the meter will display the message ***Calibration OK*** and will save the calibration data. If not, it will display an error message.
9. Measure the pH of the four buffered indicator solutions by submerging the probe in them (in the dram bottles), being careful to rinse the electrode between measurements.

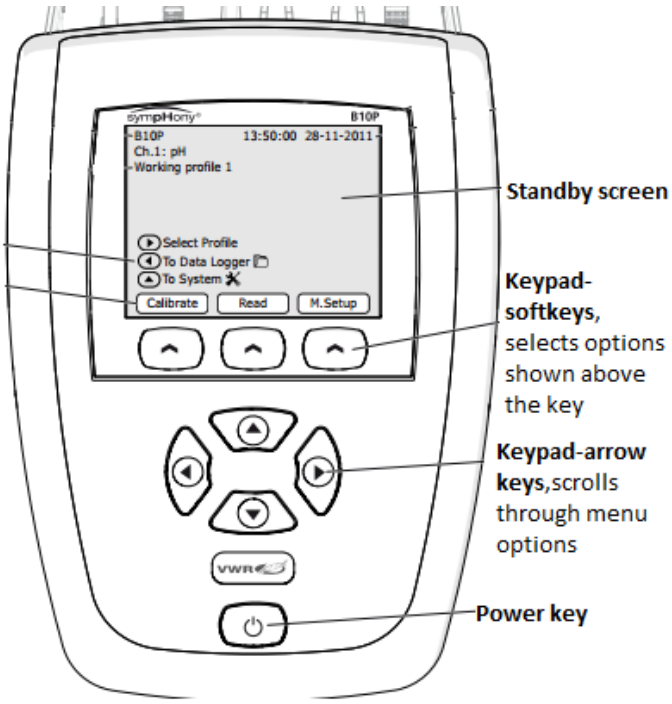
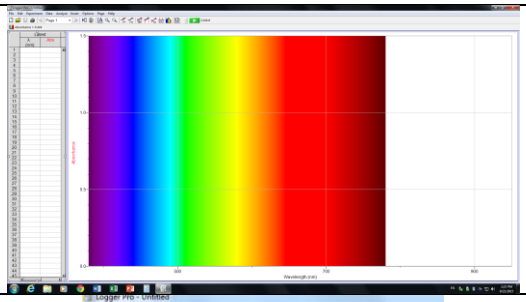
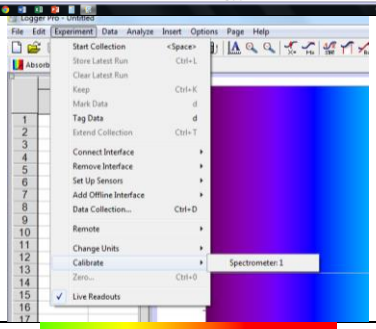
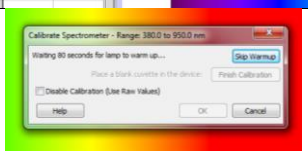
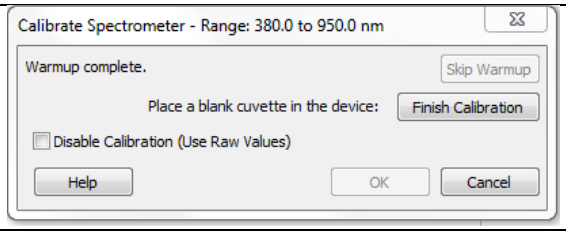


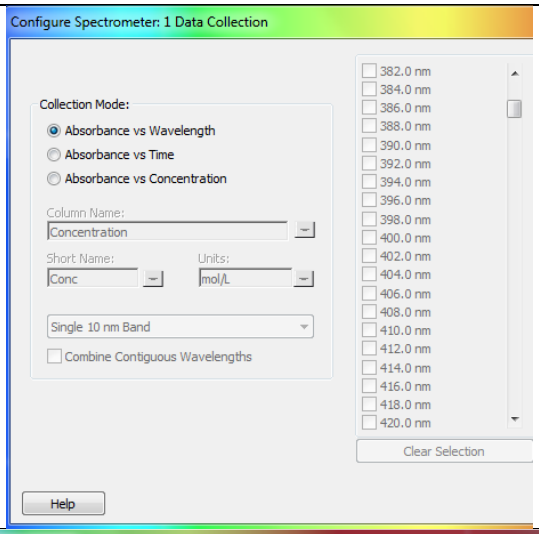
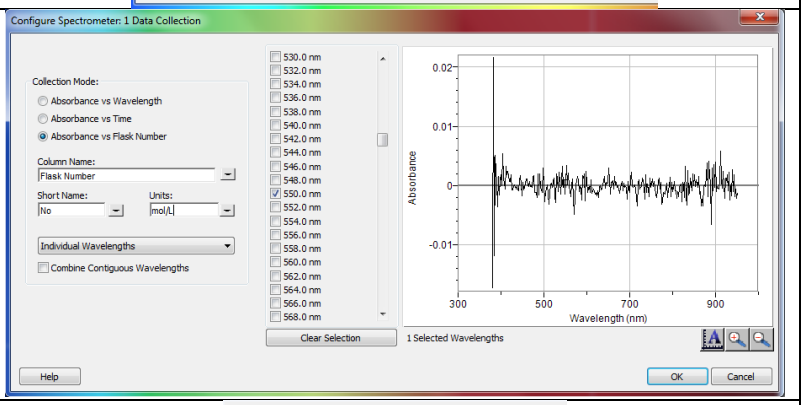
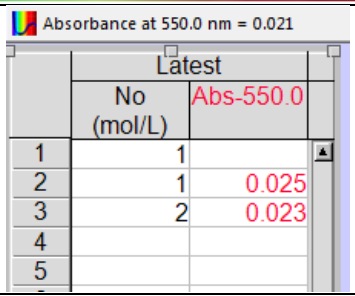
Figure 1 pH Meter Standby Screen

Absorbance Measurements

A Vernier Spectrometer will be used to take the absorbance measurement. Like the pH meter, you will need to calibrate the spectrometer before taking the measurements.

1. Open the Logger Pro software and connect the spectrometer unit to the computer USB port; you should be able to see a screen like the figure on the right. Note: For best results, allow the Spectrometer to warm up for a minimum of five minutes before proceeding.	
2. To calibrate the Spectrometer, from the Experiment menu and choose Calibrate ► Spectrometer	
3. Fill a cuvette about ¾ full with DI water to serve as the blank. Align the cuvette so that the clear side of the cuvette is facing the light source.	
4. Follow the instructions in the dialog box to complete the calibration, and then click **OK**.	

The Vernier spectrometer enables three types of data collection – absorbance (or %T) vs. wavelength, which produces a spectrum, absorbance (or %T) vs. concentration for Beer's law experiments, and absorbance (or %T) vs. time for kinetics experiments. For this experiment, we only require the absorbance at one particular wavelength (550nm), so we will use the **absorbance vs. concentration** where the concentration for our case will be taken as the different numbers of the flasks (we don't expect this to be linear, but we will process the data in Excel as described above). The following procedures are for taking the absorbance measurements.

1. Click the Configure Spectrometer Data Collection button,  .	
2.There are three regions in this box: **Collection Mode** contains three options for data collection. Choose **Absorbance vs Concentration** for this experiment. Then you need to choose a wavelength from the **List of wavelength options.** This column lists all the available wavelengths. It becomes active when either the Concentration or Time mode is selected.	
3. Select 550nm for this experiment. You can also change the column name from **Concentration** to **Flask Number** for this experiment Then Click **OK** to continue.	
4. Click ▶ Collect , then place your first sample in the cuvette slot of the Spectrometer. After the readings on the lower left corner on the screen stabilize, click ● Keep .Enter the flask number and click **OK**.	
5. Repeat Step 5 for the remaining samples.	
6. When finished, click ■ Stop to end data collection.	

REQUIRED MEASUREMENTS

Report the pH for the four buffered solutions and the absorbance of all six solutions and the derived values of log([In^{2-}]/[HIn^{-}]) for the buffered solutions. Plot pH vs. log ([In^{2-}]/[HIn^{-}]) and obtain the linear least-squares fit through the data. Note the value of pKa for Phenol Red from the measured pH values on the graph. This value should be between 7.5 and 8.0. *If not, try to identify possible calculation errors.* The graph must be presented as part of the results. If there is a difference between the measured and calculated pH values (from the PreLab calculation), suggest possibilities for its origin.

NOTES

1) The absorbance of each of the compounds (HIn^{-} and In^{2-}) acts independently and we see the sum of the absorbances of the two compounds present. The absorbance is what makes the solution appear colored.

Beer's Law: $A = A(HIn^{-}) + A(In^{2-}) = \varepsilon(HIn^{-})\, b\, c(HIn^{-}) + \varepsilon(In^{2-})\, b\, c(In^{2-})$

2) We recommend adding the indicator to the flasks first for two reasons: 1) if after adding the indicator to the flask it turns orange, the flask has not been cleaned properly and you will need to clean and rinse it and try again; and 2) people sometimes forget to add the indicator if they don't do it first.

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