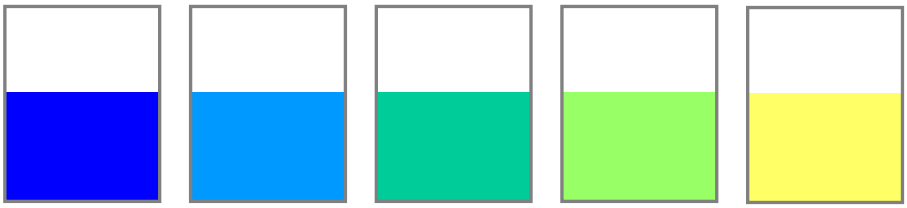
Spectrometric Determination of the Acid Dissociation Constant of an Acid-base Indicator

Learning Goals

1. Gain appreciation of the dynamics of perturbing a chemical equilibrium
2. Gain an understanding of how to use Beer's Law, especially in conjunction with a two-component mixture.
3. Practice preparing standard solutions for producing Beer's law plots.
4. Gain a deep appreciation for how acid-base indicators work.
5. Distinguish between directional error and random uncertainty.

Introduction

In this experiment you will determine the acid dissociation constant, K_a, of an acid-base indicator system. An acid base indicator is generally a weak acid-base system, HIn/In^-, where the HIn form is a different color than the In^- form in aqueous solutions. As a result an aqueous solution of an indicator goes through a color transition with in a fairly narrow pH range, $\Delta ph = 2$. As an example, the illustration below represents a solution of the indicator, as the pH increases from pK_a -1 to pK_a+1. In this illustration the acidic form (HIn) is blue and basic form (In^-) is yellow.



Each of these beakers contains a solution of the same four substances at a different pH. You will study a similar set of solutions in this experiment. Your set of solutions will contain different substances and will be different colors from those shown here.

The K_a for an indicator system is the equilibrium constant for the following reaction.

$$HIn\ (aq) + H_2O\ (l) \leftrightarrows In^-\ (aq) + H_3O^+\ (aq) \qquad \text{(rxn 1)}$$

$$Ka = \frac{[In^-][H_3O^+]}{[HIn]}$$

In this experiment you will begin with a dilute solution of an indicator buffered at a pH near its pKa. You will measure the concentrations of $[In^-]$, [HIn] and $[H_3O^+]$ and use these concentrations to calculate a

value for K_a. Details of these measurements are discussed below. Then you will add a little HCl to the solution. The HCl will drive the reaction to the left (according to Le Chatelier's Principle). You will remeasure the concentrations of [In^-], [HIn] and [H_3O^+] and use these concentrations to calculate another value for K_a. You will repeat this process until you have several measurements of K_a. Next, you will add a little NaOH to the solution. The NaOH will consume some H_3O^+ and drive rxn 1 to the right (according to Le Chatelier's Principle). You will remeasure the concentrations of [In^-], [HIn] and [H_3O^+] and use these concentrations to calculate another value for K_a. You will repeat this process until you have made several additional measurements of K_a. In the end you should have about twelve measurements in a pH range of 1.4 bracketing the expected pK_a of the indicator.

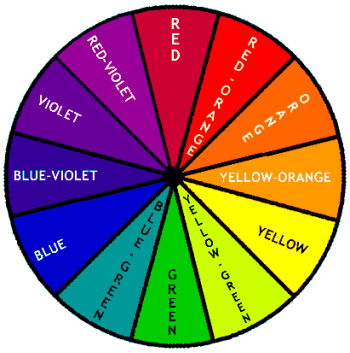
Measuring [H_3O^+]

For each solution the [H_3O^+] will be determined by measuring the pH using a pH meter. We will then use the pH to calculate [H_3O^+]. We will learn how to properly use a pH meter, including how to use buffers to calibrate, the importance of thoroughly rinsing and blot drying the probe between measurements and the importance of periodically recalibrating to avoid drift issues.

Spectroscopic measurement of [HIn] and [In^-]

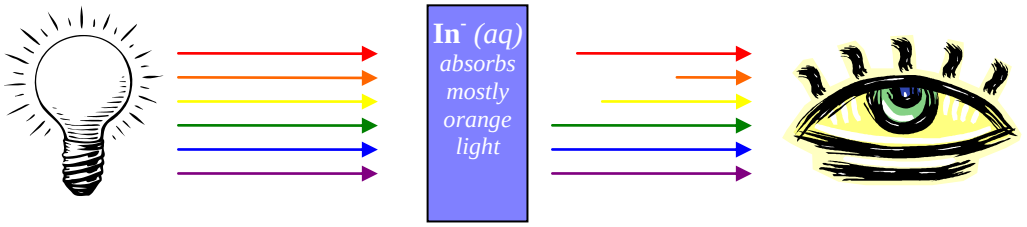
$HIn_{(aq)}$ is a different color than $In^-_{(aq)}$, while water and the hydronium ion, $H_3O^+_{(aq)}$, are colorless. So the color of the solution in which this reaction is happening depends on the ratio $HIn_{(aq)}/In^-_{(aq)}$. For example, if $HIn_{(aq)}$ is yellow and $In^-_{(aq)}$ is blue, then a solution that is yellow must contain far more $HIn_{(aq)}$ than $In^-_{(aq)}$. On the other hand, a blue solution contains a lot more $In^-_{(aq)}$ than $HIn_{(aq)}$. And a green solution would contain a nearly equal mixture of $HIn_{(aq)}$ and $In^-_{(aq)}$, because green is the color that results when yellow and blue are mixed equally (this is the situation when the pH = pKa of the indicator). So, it is possible to estimate the relative amount of $HIn_{(aq)}$ and $In^-_{(aq)}$ just by looking at the solution. However, part of this experiment involves measuring the amount of $HIn_{(aq)}$ and $In^-_{(aq)}$ more precisely than is possible using just your eyes. The instrument that we will use to measure the color of the solution is a **spectrophotometer**.

White light, the light that we are all familiar with, is a blend of all colors of light in the visible spectrum. When the colors of light are separated they can form a rainbow. A spectrophotometer separates light into its separate colors. It is able to separate the light into colors because each color of light has a different wavelength than the other colors. The spectrophotometer can shine a narrow band of



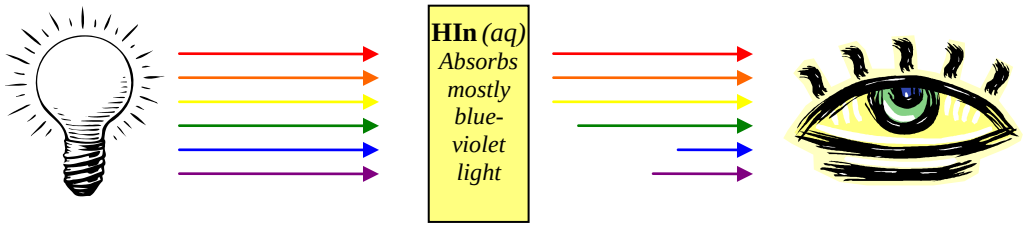
Color wheel needed for pre-lab question 2(c).

wavelengths of light on a sample and then measure how much of that light is absorbed by the sample. Different colored substances absorb varying amounts of specific wavelengths of light. Therefore, a spectrophotometer can be used to measure how much of a substance is present. The color that a substance appears to your eye is a consequence of the colors of light that the substance does not absorb. In other words, substances absorb most strongly colors of light that are complementary to the color that they appear. For example, if $In^-_{(aq)}$ is blue, it would absorb a lot of light that had a wavelength of 640nm (orange light), but very little light that had a wavelength of 430 nm (blue-violet light), as shown in the diagram below.



White light, containing all colors, shines on In^-. In^- absorbs most of the orange light. The remaining colors of light blend together, appearing blue to your eyes.

On the other hand, a sample that contains mostly yellow HIn would do the opposite – it would absorb very little orange 640 nm light and a lot of blue-violet 430 nm light, as shown below.



White light shines on HIn. HIn absorbs most of the blue-violet light. The remaining colors of light blend together, appearing yellow to your eyes.

A solution that contains both HIn and In^-, and which has a high absorbance at 430 nm and a low absorbance at 640 nm indicates that the solution absorbs little orange light and a lot of blue-violet light. Therefore, that solution contains a larger amount of HIn and smaller amount of In^-. In order to measure the amount of HIn and In^- very precisely, you will convert the absorbances measured using the spectrophotometer to the [HIn] and [In^-] in the solutions using a **calibration curve**. A calibration curve is a graph of **absorbance**, how much of a particular wavelength of light is absorbed, versus concentration (Beer's law). A calibration curve is specific to a particular substance, and must be created by measuring the absorbance of a few solutions of known concentration.

Beer's Law Plots

In a mixture of HIn and In^- the absorbance at a particular wavelength is the sum of the absorbances of HIn and In^- at that wavelength.

$$A(\lambda_1) = A(\lambda_1)_{HIn} + A(\lambda_1)_{In^-}$$

In this experiment we will be measuring the absorbance of each solution at two different wavelength; one where HIn absorbs strongly and another where In- absorbs strongly. But, as seen below from some of the spectra shown below, the visible absorption spectra of HIn and In- are quite broad. As a result, absorbances from both components tend to be significant at all wavelengths. In general, we can determine the concentration of two absorbing species in a mixture by measuring the absorbance of the mixture at two different wavelengths and by obtaining calibration curves for both components at both wavelengths. With this in hand one can construct and solve a system of two independent equations that contain the two unknown concentrations, [HIn] and $[In^-]$, in the mixture.

$$A(\lambda_1) = A(\lambda_1)_{HIn} + A(\lambda_1)_{In^-} = e_{HIn@\lambda1}b[HIn] + e_{In^-@\lambda1}b[In^-]$$
$$A(\lambda_2) = A(\lambda_2)_{HIn} + A(\lambda_2)_{In^-} = e_{HIn@\lambda2}b[HIn] + e_{In^-@\lambda2}b[In^-]$$

Here, $e_{HIn@\lambda1}b$ represents the slope of the Beer's law plot for the HIn species at λ_1 and $e_{HIn@\lambda1}b$ represents the slope of the Beer's law plot for the HIn species at λ_2. These Beer's Law plots will be produced by using standard solutions prepared at a pH that is three pH units less than the pKa of the indicator, so that 99.9% of the indicator is in the HIn form. Likewise, $e_{In^-@\lambda1}b$ represents the slope of the Beer's law plot for the In^- species at λ_1 and $e_{In^-@\lambda2}b$ represents the slope of the Beer's law plot for the In^- species at λ_2. These Beer's Law plots will be produced by using standard solutions prepared at a pH that is three pH units higher than the pKa of the indicator, so that 99.9% of the indicator is in the In^- form.

The concentrations of [HIn] and $[In^-]$ can be calculated by solving the system of equations, where s is used to represents the slopes of the four Beer's Law plots:

$$A(\lambda_1) = s_{HIn@\lambda1}[HIn] + s_{In-@\lambda1}[In^-]$$

$$A(\lambda_2) = s_{HIn@\lambda2}b[HIn] + s_{In-@\lambda2}[In^-]$$

Solving this system of equations is not difficult, and you can earn extra credit for turning in a step by step solution of the problem, separately from your report, to Professor Evans, during the next discussion section. Below are the final solutions.

$$[In-] = (s_{HIn@\lambda2}\, A(\lambda_1) - s_{HIn@\lambda1}\, A(\lambda_2)) / (s_{HIn@\lambda2}s_{In-@\lambda1} - s_{HIn@\lambda1}s_{In-@\lambda2})$$

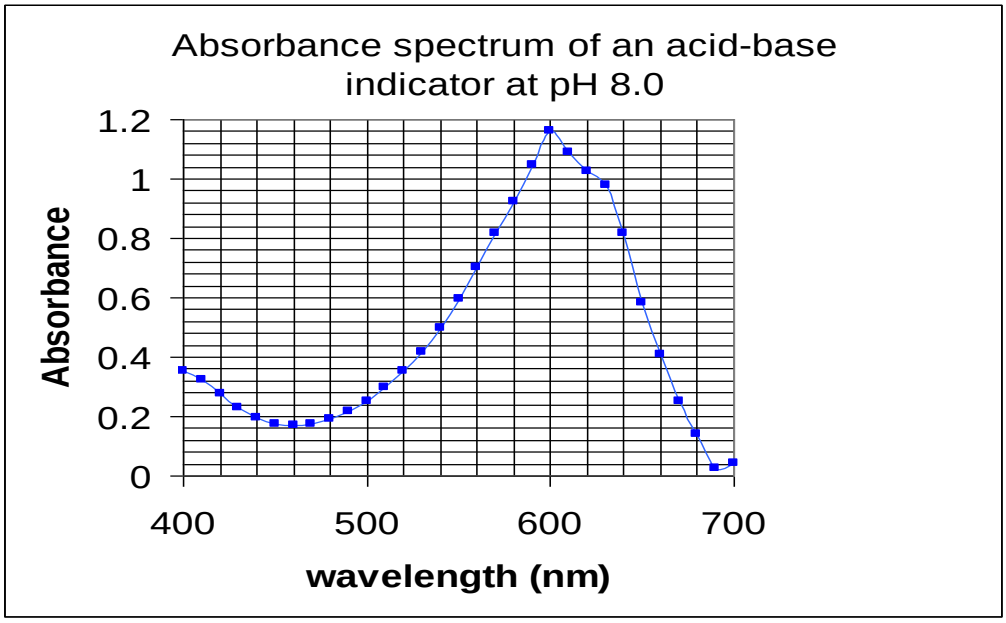
$$[HIn] = (A(\lambda_1) - \{slope\}_{In-@\lambda1}[In^-])/\{slope\}_{HIn@\lambda1}$$

One final note: Generally, the wavelengths are chosen so that the ratios between the absorbances of the two species, A_{HIn} / A_{In-}, are maximized and minimized. The wavelength chosen in this write-up reflect the limitations of some fix wavelength spectrometers that were used previously in this course.

Prelab Questions

1. A certain indicator is red in its HIn *(aq)* form and yellow in its In^- *(aq)* form. What color would you expect the following solutions to appear? **Explain why**.
   a) A 1:1 HIn *(aq)*:In^- *(aq)* mixture?
   b) A 1:100 HIn *(aq)*:In^- *(aq)* mixture?
   c) A 3:1 HIn *(aq)*:In^- *(aq)* mixture?

2. The absorbance spectrum of a substance is a graph of wavelength versus absorbance. Study the absorbance spectrum shown below.
   a) The symbol for wavelength is λ, and the wavelength at which a substance absorbs the most light is λ_{max}. What is λ_{max} for the substance whose spectrum is shown below?



   b) Use the electromagnetic spectrum in your text book to determine the color of light that corresponds to λ_{max}.
   c) Based on λ_{max}, what color would you expect this substance to appear to your eye? (HINT—the color is opposite λ_{max} on the color wheel on page 2 of this lab write-up.)

Procedure

Instructions and Overview

You will be assigned to study one indicator, either bromophenol blue or phenol red. You are going to make an aqueous solution of this acid-base indicator. You will calibrate the equipment, a pH meter and a spectrophotometer, that you need to use for the experiment. Next you will prepare a series of HIn standards and a series of In- standards that will be used to produce the four beer's law plots. Finally, you will prepare an indicator solution near the pKa of the indicator, measure its pH and its absorbance at both wavelengths. Using dilute solutions of NaOH and HCl you will adjust the pH and remeasure the absorbances to several times.

You will use the excel template associated with this experiment to process your data.

Procedure and Calculations Flow Chart

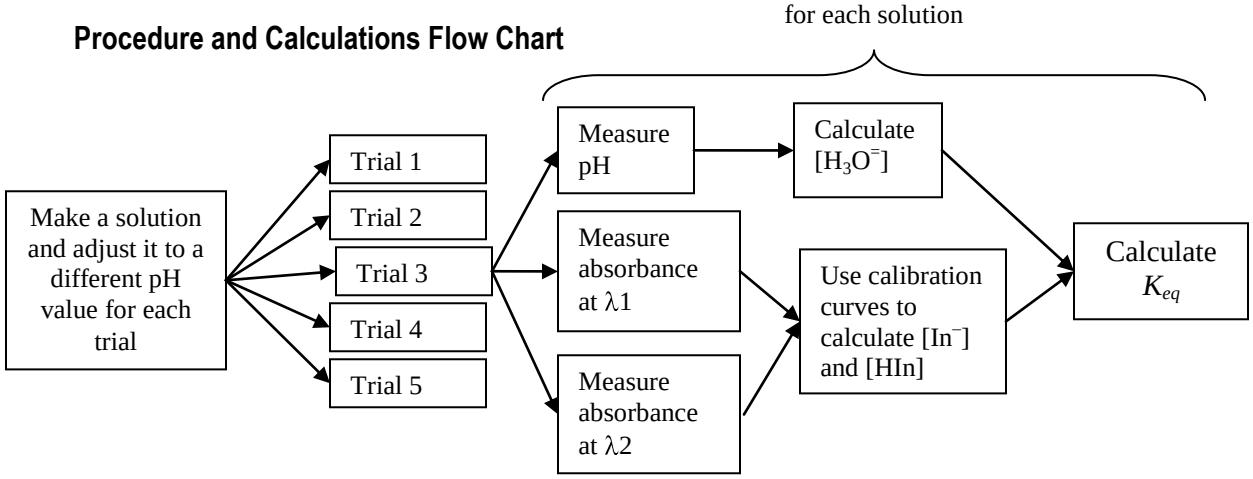


Table 1 -- pH and wavelength settings for different indicators			
Assigned indicator	**pH values (adjust one trial to each pH value)**	**Absorbance ratio maximum for HIn (λ_1)**	**Absorbance ratio maximum for In^- (λ_2)**
Bromophenol blue	3.3 -4.7	430	565
Phenol red	7.0-8.4	430	565

Preparing the standards

Bromophenol Blue

Master HIn standard: std 1 has been prepared for you. Its concentration is $2.985 \cdot 10^{-5}$ M. Take about 10 mL of std 1. Prepare std 2 by mixing 3.00 mL of std 1 and 1.00 mL of 0.10 M HCl using a micropipette. Prepare std 3 by mixing 2.00 mL of std 1 and 2.00 mL of 0.10 M HCl using a micropipette. Prepare std 4 by mixing 1.00 mL of std 1 and 3.00 mL of 0.10 M HCl using a micropipette. Prepare std 5 by mixing 1.00 mL of std 3 and 3.00 mL of 0.10 M HCl using a micropipette.

Master In^- standard: std 1 has been prepared for you. Its concentration is $2.985 \cdot 10^{-5}$ M. Take about 10 mL of std 1. Prepare std 2 by mixing 3.00 mL of std 1 and 1.00 mL of diluted buffer 7 using a micropipette. Prepare std 3 by mixing 2.00 mL of std 1 and 2.00 mL of diluted buffer 7 using a micropipette. Prepare std 4 by mixing 1.00 mL of std 1 and 3.00 mL of diluted buffer 7 using a micropipette. Prepare std 5 by mixing 1.00 mL of std 3 and 3.00 mL of diluted buffer 7 using a micropipette.

Phenol Red

Master HIn standard: std 1 has been prepared for you. Its concentration is $2.882 \cdot 10^{-5}$ M. Take about 10 mL of std 1. Prepare std 2 by mixing 3.00 mL of std 1 and 1.00 mL of diluted buffer 4 using a micropipette. Prepare std 3 by mixing 2.00 mL of std 1 and 2.00 mL of diluted buffer 4 using a micropipette. Prepare std 4 by mixing 1.00 mL of std 1 and 3.00 mL of diluted buffer 4 using a micropipette. Prepare std 5 by mixing 1.00 mL of std 3 and 3.00 mL of diluted buffer 4 using a micropipette.

Master In^- standard: std 1 has been prepared for you. Its concentration is $2.882 \cdot 10^{-5}$ M. Take about 10 mL of std 1. Prepare std 2 by mixing 3.00 mL of std 1 and 1.00 mL of diluted buffer 10 using a micropipette. Prepare std 3 by mixing 2.00 mL of std 1 and 2.00 mL of diluted buffer 10 using a micropipette. Prepare std 4 by mixing 1.00 mL of std 1 and 3.00 mL of diluted buffer 10 using a micropipette. Prepare std 5 by mixing 1.00 mL of std 3 and 3.00 mL of diluted buffer 10 using a micropipette.

Measuring absorbances of the standards: Measure the absorbances of the five HIn standards and the five In^- standards at both wavelengths. (see calibrating the spectrophotometer)

Preparing the testing solution

If you were assigned bromophenol blue, mix 1 mL of the commercial bromophenol blue indicator, 0.5 mL of diluted buffer 4 and add about 10 mL of de-ionized water in a 20 mL screw cap vial. If you were assigned phenol red, mix 1 mL of the commercial phenol red indicator, 0.5 mL of diluted buffer 7 and add about 10 mL of de-ionized water in a 20 mL screw cap vial.

Calibrating the spectrophotometer

Each row, which is shared by two pairs of students, will have two spectrophotometers. One of these will be dedicated to measuring the absorbances of solutions at 430 nm and the other will be dedicated to measuring the absorbances of solutions at 565 nm. This will prevent the issue of having to set the 100 % transmittance using the blank when switching wavelengths, which can easily cause some "careless" errors. Each spectrophotometer will have a cuvette filled with de-ionized water to use as the blank to set the 100 % T, but the wavelength will not have to be switched back and forth.

Calibrating the pH meter

Instructors will demonstrate.

Adjusting and measuring the pH of the solution

1. Immerse the pH electrode in the solution.
2. Wait for the pH reading to stabilize. Then record the exact pH in your notebook.
3. Using a Pasteur Pipette place a portion of the solution in the cuvette and measure and record the absorbances at 430 nm and 565 nm (see above)
4. Empty the contents of the cuvette back into the beaker and add 0.1 M HCl or 0.1 M NaOH solution, depending on whether you are trying to make the solution more acidic or more basic, dropwise until the pH changes by at least 0.10 pH units.
5. Using a Pasteur Pipette rinses your cuvette with the solution several times, each time pouring the contents back into the beaker. Finally, place a portion of the solution in the cuvette and measure and record the absorbances at 430 nm and 565 nm. Record the pH of the solution.

6. Go to step 4 and repeat the process until you have twelve different sets of measurements of pH and the absorbances at both wavelengths at pH values roughly equally spaced between 3.3 and 4.7 for bromophenol blue or between 7.0 and 8.4 for phenol red.

Data Section of Lab Report ; {to be included in your lab report (word file)}

1. Show the four Beer's Law Plots (3 pt each) on a single graph and produce a table that reports the slopes of these plots. (5 pts) Are the intercepts near zero, as expected? If not, how should you proceed?
2. Show a table of [HIn], [In^-], [H_3O^+], K_a and pK_a values determined for each of your test solutions. (5 pts) These values of [HIn] and [In^-] are calculated on your spreadsheet, using the slopes of the Beer's Law Plots and the solution to the set of equations. The [H_3O]$^+$ is calculated from the measured pH.
3. Calculate the alpha fractions of HIn and In- from [HIn] and [In^-] for each of you twelve test solutions and plot these alpha fractions as a function of pH on the same plot. (5 pts) Estimate the pK_a of your indicator from this plot. (3 pts)
4. Plot the calculated pK_a values as a function of the measured pH value for your twelve measurements. (5 pts) This plot will be used to address #3 in the discussion section.
5. Calculate your mean value of K_a and report the K_a as mean ± 95 % CI. (5 pts) Calculate your mean value of pK_a and report the pK_a as mean ± 95 % CI. (5 pts)
6. The literature value of K_a for bromophenol blue is 1.15×10^{-4}. The literature value of K_a for phenol red is 2×10^{-8}. Calculate the percent error of your mean K_a value using this equation: (5 pts)

$$\%\,error = \frac{\left|\text{literature value} - \text{experimental value}\right|}{\text{literature value}} \times 100\%$$

Discussion Section

Analyze the plot in #3 carefully. Discuss if [HIn] and [In^-] changed as a function of pH in a predictable way, according to Le Chatelier's principle. (5 pts)

Why did we need four Beer's Law Plots in this experiment? (5 pts)

Discuss the possible factors that are contributing to the uncertainty in K_a. Does the variation in the calculated pK_a values appear to be random or is it a function of pH? If it appears to be a function of pH, discuss the possible reasons and the implications. (5 pts)

Discuss the possible factors contributing to the % error in the measurement. (5 pts)

Lab Report

SPREADSHEET	10
ABSTRACT	10
PROCEDURE	10
DATA	50
DISCUSSION	20