Spectrometric Determination of the Acid Dissociation Constant of an Acid-base Indicator
Experiment 3

Introduction

In this experiment you will study a chemical reaction by observing and measuring the color of several different solutions. Each solution you study will be at a different pH and will be a different color, but all of the solutions will contain the same four substances interacting chemically with each other in a similar way. You will use the differences in pH and color to understand what is going on chemically in each solution.

The colored substances that you will be studying are two different molecules which differ from each other by only a hydrogen ion (H⁺). One of the molecules, which has the symbol HIn, is chemically converted to the other the molecule, which has symbol In⁻, when it reacts with water in solution as shown in the equation below. (Note that “In” stands for indicator, and H is hydrogen. HIn is a weak acid, and In⁻ is its conjugate base.)

\[
\text{HIn} \ (aq) + \text{H}_2\text{O} \ (l) \rightleftharpoons \text{In}^- \ (aq) + \text{H}_3\text{O}^+ \ (aq)
\]

HIn \ (aq) is a different color than In⁻ \ (aq), while water and the hydronium ion H₃O⁺ \ (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:

\[
\text{HIn} \ (aq) + \text{H}_2\text{O} \ (l) \rightleftharpoons \text{In}^- \ (aq) + \text{H}_3\text{O}^+ \ (aq)
\]

Yellow \quad \text{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 $\text{HIn} \ (aq)$ and $\text{In}^- \ (aq)$ 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 wavelengths (essentially one specific color) of light on a sample of substance 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 $\text{In}^-$ $(aq)$ is blue, it would absorb a lot of light that had a wavelength of 640 nm (orange light), but very little light that had a wavelength of 430 nm (blue-violet light), as shown in the diagram below.

On the other hand, a sample that contains mostly yellow $\text{HIn}$ 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.
A solution that contains both HIn and In\textsuperscript{−}, 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\textsuperscript{(aq)} and smaller amount of In\textsuperscript{−}\textsuperscript{(aq)}. In order to measure the amount of HIn\textsuperscript{(aq)} and In\textsuperscript{−}\textsuperscript{(aq)} very precisely, you will convert the spectrophotometer readings you take in the lab to the molar concentration of HIn\textsuperscript{(aq)} and In\textsuperscript{−}\textsuperscript{(aq)} 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 large number of solutions of known concentration. You do not have to create your own calibration curves in this experiment.

NOTE: The calibration curves were produced by measuring dilute solutions of the indicator at various known concentrations at pH = pKa + 3 for In\textsuperscript{−} and at pH = pKa - 3 for HIn. Working at pH values removed from the 3 pH units removed from the pKa ensured that we the standards contained essentially all In\textsuperscript{−} at pKa + 3 and all HIn at pKa − 3. At these pHs essentially all of the indicator is in the In\textsuperscript{−} and HIn forms, respectively.

In a mixture of HIn and In\textsuperscript{−} the absorbance at a particular wavelength is the sum of the absorbances of HIn and In\textsuperscript{−} at that wavelength.

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

where \(s_{HIn@\lambda_1}\) and \(s_{In^{-}@\lambda_1}\) are the slopes of the Beer’s law plots.

As seen below from some of the spectra shown below, the visible absorption spectra of HIn and In\textsuperscript{−} are quite broad. As a result, absorbances from both components tend to be significant at both low and high 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.

\[
A(\lambda_1) = s_{HIn@\lambda_1}[HIn] + s_{In^-@\lambda_1}[In^-] \\
A(\lambda_2) = s_{HIn@\lambda_2}[HIn] + s_{In^-@\lambda_2}[In^-]
\]

Generally, the wavelengths are chosen so that the ratios between the absorbances of the two species, \(A_{HIn} / A_{In^-}\), are maximized and minimized. You will be given the four values for the slopes from the four calibration curves that were prepared in advance. You will adjust the pH of your dilute indicator solution incrementally around the pH = pKa ± 0.5 and at each increment (5 increments in all) measure the absorbance of the solution at both wavelengths, \(A(\lambda_1)\) and \(A(\lambda_2)\).

Solving this system of equations is not difficult, but because of our limited time the solution is provided below.

\[
[In^-] = (s_{HIn@\lambda_2} A(\lambda_1) - s_{HIn@\lambda_1} A(\lambda_2)) / (s_{HIn@\lambda_2} s_{In^-@\lambda_1} - s_{HIn@\lambda_1} s_{In^-@\lambda_2}) \\
[HIn] = (A(\lambda_1) - \{slope\}_{In^-@\lambda_1}[In^-]) / \{slope\}_{HIn@\lambda_1}
\]

After you determine the concentration of HIn (aq) and In^- (aq) using the spectrophotometer and and the slopes in Table 2, you will measure the pH of your solutions with a pH meter. pH is a measure of the amount of hydronium ion, \(H_3O^+ (aq)\) in a solution; pH is equal to the negative logarithm of the hydronium ion concentration:

\[
pH = -\log[H_3O^+]
\]

Mathematically the above equation translates to:

\[
[H_3O^+] = 10^{-pH}
\]

Once you know the concentrations of HIn (aq), In^- (aq) and \(H_3O^+ (aq)\) in your colored solutions, you can use that information to describe the solutions numerically by calculating the equilibrium constant, \(K_a\), and the pKa using the data from each solution.
One of the main goals of this experiment is to calculate the equilibrium constant for each of your solutions as accurately as possible. Remember that the reaction you are studying is:

$$\text{HIn} (aq) + \text{H}_2\text{O} (l) \rightleftharpoons \text{In}^- (aq) + \text{H}_3\text{O}^+ (aq)$$

The equilibrium constant for this reaction is calculated by multiplying the concentrations of the two products, $\text{In}^- (aq)$ and $\text{H}_3\text{O}^+ (aq)$, and dividing by the concentration of the reactant $\text{HIn} (aq)$ as shown below:

$$K_a = \frac{[\text{In}^-][\text{H}_3\text{O}^+]}{[\text{HIn}]}$$

Recall that $\text{H}_2\text{O} (l)$ does not appear in the above equilibrium constant expression.

Once you have calculated the $K_a$ for all of your solutions, you will use those values to understand and describe the chemical reaction.
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 \( \lambda \), and the wavelength at which a substance absorbs the most light is \( \lambda_{\text{max}} \). What is \( \lambda_{\text{max}} \) for the substance whose spectrum is shown below?

   ![Absorbance spectrum of an acid-base indicator at pH 8.0](image)

   b) Use the electromagnetic spectrum in your text book (e.g., Kotz & Treichel, p. 256) to determine the color of light that corresponds to \( \lambda_{\text{max}} \).

   c) Based on \( \lambda_{\text{max}} \), what color would you expect this substance to appear to your eye? (HINT—the color is opposite \( \lambda_{\text{max}} \) on the color wheel on page 2 of this lab write-up.)

3. The pH of a bromophenol blue solution containing a mixture of yellow HIn (aq) and blue In⁻ (aq) molecules is 3.17. See Table 1 for data on the slopes of the calibration curves.
   a) What is the hydronium ion concentration, \([H_3O^+]\), of this solution?
   b) The solution has absorbance 0.336 at 430nm and an absorbance 0.143 at 590nm determine \([\text{In}^-]\) and \([\text{HIn}]\). Using your answers to parts a and b calculate \( K_{eq} \) for this solution.
   c) What color would you expect this solution to appear?
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 take some of the solution you made and adjust it to a particular pH. Then you will measure the % transmittance of the solution at two different wavelengths. After doing this you will adjust the solution you made to a different pH, and record its % transmittance at both wavelengths. You will repeat this four more times at different pH values. You will convert the %transmittance measurements to concentration using calibration curves and use these results and the measured pH to determine the equilibrium constant ($K_{eq}$) for each of the solutions.

The details on the pH range and wavelengths to use are found in Table 1 and detailed step-by-step instructions for each part of the experiment are below. The Procedure and Calculations Flow Chart below illustrates the overall process you will carry out.

<table>
<thead>
<tr>
<th>Table 1 -- pH and wavelength settings for different indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assigned indicator</strong></td>
</tr>
<tr>
<td>Bromophenol blue</td>
</tr>
<tr>
<td>Phenol red</td>
</tr>
</tbody>
</table>

Procedure and Calculations Flow Chart

- Make a solution and adjust it to a different pH value for each trial
- Measure pH
- Measure absorbance at $\lambda_1$
- Measure absorbance at $\lambda_2$
- Calculate $[\text{H}_3\text{O}^+]$
- Use calibration curves to calculate $[\text{In}^-]$ and $[\text{HIn}]$
- Calculate $K_{eq}$

for each solution
***IMPORTANT NOTE***
All measurement readings on the spectrophotometer must be done in terms of % transmittance (the top scale on the dial). Later on, you will mathematically convert your transmittance readings into absorbance, as described in the Calculations section of this handout. The reason we read in transmittance and convert to absorbance later is because absorbance is a logarithmic scale, while transmittance is a linear scale. Basically, this means that it is much easier to accurately read transmittance than it is to accurately read absorbance.

### Table 2 – Slopes from standard curves

<table>
<thead>
<tr>
<th>Assigned indicator</th>
<th>$s_{HIn@430}$</th>
<th>$s_{In@430}$</th>
<th>$s_{HIn@565}$</th>
<th>$s_{In@565}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromophenol blue</td>
<td>14463</td>
<td>3532</td>
<td>48</td>
<td>25614</td>
</tr>
<tr>
<td>Phenol red</td>
<td>15318</td>
<td>4851</td>
<td>0</td>
<td>15434</td>
</tr>
</tbody>
</table>

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Making the acid base-indicator solution
1. If you were assigned bromophenol blue, mix 300 μL of the bromophenol blue indicator and 1 mL of buffer 4 in a 20 mL small plastic screw cap vial. Add about 10 mL of water. If you were assigned phenol red, mix 300 μL of the phenol red indicator and 1 mL of buffer 4 in a 20 mL small plastic screw cap vial. Add about 10 mL of water.

Calibrating the spectrophotometer
Instructors will demonstrate.

Calibrating the pH meter
Instructors will demonstrate.

Adjusting and measuring the pH of the solution
1. Immerse the pH electrode in the solution in the plastic tube.
2. Wait for the pH reading to stabilize. Then record the exact pH in your data table.
3. Using a Pasteur Pipette place a portion of the solution in the cuvette and measure the absorbances at 430 nm and 565 nm (see below)
4. Add a drop or two of 0.1 M HCl or 0.1 M NaOH solution, depending on whether you need to make the solution more acidic or more basic, to the solution in the tube. Put the top on and shake well.
5. Measure the pH. Wait for the pH reading to stabilize. Then record the exact pH in your data table.

6. Go to step 3 and repeat the process until you have measurements of pH and the absorbances at 10 pH values between 3.5 and 4.5 for bromophenol blue and 7.0 and 8.0 for phenol red.

Reading the % transmittance of the solution at 430 nm and 565 nm

1. Remove the blank from the spectrophotometer and insert the cuvet containing your solution. Wait for the % transmittance reading to stabilize, then record it in your data table.

2. Recalibrate the spectrophotometer (see calibrating the spectrophotometer above) to the second wavelength.

3. Save the cuvet containing your solution in a test tube rack.

4. Dump the solution in the cuvet back into the tube and adjust the pH to the next desired value (step 4 above).

Data Table

<table>
<thead>
<tr>
<th>Trial</th>
<th>Color</th>
<th>pH</th>
<th>% Transmittance at 430 nm</th>
<th>% Transmittance at 565 nm</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>5</td>
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</tbody>
</table>

Assigned indicator:

EQUIPMENT TO USE:
- Your eyes
- pH meter
- Spectrophotometer at 430 nm
- Spectrophotometer at 565 nm
Calculations
Use your data, the equations below, and the calibration curves for HIn and In\(^-\) to complete the calculations.

\[
\text{HIn} \ (aq) + \ H_2O \ (l) \ \rightleftharpoons \ \text{In}^- \ (aq) + \ H_3O^+ \ (aq)
\]

Absorbance \(= 2 - \log (\text{% transmittance})\)

\[
[H_3O^+] = 10^{-\text{pH}}
\]

\[
K_a = \frac{[\text{In}^-][H_3O^+]}{[\text{HIn}]}
\]

\[
pK_a = -\log(Ka)
\]

<table>
<thead>
<tr>
<th>Trial</th>
<th>Abs at 430 nm</th>
<th>Abs at 565 nm</th>
<th>$[H_3O^+]$</th>
<th>$[\text{HIn}]$</th>
<th>$[\text{In}^-]$</th>
<th>$K_a$</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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</tr>
</tbody>
</table>
**Literature comparison**

1. Calculate your mean value of $K_a$ by averaging the $K_a$ values for your five solutions. Calculate a standard deviation and report the $K_a$ as value ± stand dev.

2. The literature value of $K_a$ for bromophenol blue is $1.15 \times 10^{-4}$. The literature value of $K_a$ for phenol red is $3.5 \times 10^{-8}$. Calculate the percent error of your mean $K_a$ value using this equation:

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

**Post-lab questions and analysis**

1. Why are the solutions in each of your cuvettes different colors?

2. Compare the $K_a$ values of phenol red and bromphenol blue. 
   a. Which is larger?
   b. What do the different $K_a$ values tell you about these two reactions as compared to one another?
3. Why does the procedure call for groups who studied phenol red to adjust the pH of their solutions to different values (between 7.0 and 8.0) than the groups who studied bromphenol blue (3.5 and 4.5)?

4. Compare these two solutions in terms of pH:

   Phenol red in water with \([\text{HIn}] = [\text{In}^-] = 0.000050\text{M}\)
   Bromphenol blue in water with \([\text{HIn}] = [\text{In}^-] = 0.000050\text{M}\)
   
   a. Which has a larger pH value?
   
   b. Why must they have different pH values?

5. A cation of a metal (A) and an anion (B) react to form a soluble complex whose formula is unknown. The formula of the complex could be \(\text{AB}, \text{A}_2\text{B}, \text{or AB}_2\).

   Use the data set and equations below to determine the formula of the complex.

<table>
<thead>
<tr>
<th>Concentration of cation of A (M)</th>
<th>Concentration of anion of B (M)</th>
<th>Concentration of soluble complex of A and B (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1.09 \times 10^{-1})</td>
<td>(9.56 \times 10^{-1})</td>
<td>(3.56 \times 10^{-3})</td>
</tr>
<tr>
<td>(3.24 \times 10^{-3})</td>
<td>(5.60 \times 10^{-5})</td>
<td>(1.84 \times 10^{-10})</td>
</tr>
<tr>
<td>(5.25 \times 10^{-1})</td>
<td>(5.63 \times 10^{-1})</td>
<td>(4.86 \times 10^{-2})</td>
</tr>
<tr>
<td>(5.60 \times 10^{-4})</td>
<td>(1.20 \times 10^{-7})</td>
<td>(1.18 \times 10^{-14})</td>
</tr>
<tr>
<td>(5.2 \times 10^{-3})</td>
<td>(1.83 \times 10^{-1})</td>
<td>(1.55 \times 10^{-6})</td>
</tr>
</tbody>
</table>

If the formula of the complex is \(\text{AB}\) then \(K_{eq} = \frac{[\text{AB}]}{[\text{A}^+] [\text{B}^-]}\)

If the formula of the complex is \(\text{A}_2\text{B}\) then \(K_{eq} = \frac{[\text{A}_2\text{B}]}{[\text{A}^+]^2 [\text{B}^2-]}\)

If the formula of the complex is \(\text{AB}_2\) then \(K_{eq} = \frac{[\text{AB}_2]}{[\text{A}^{2+}] [\text{B}^-]^2}\)

What is the formula of the complex? Show work to support your answer.