What Can Be Measured in a Colorful Equilibrium Reaction?

**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.

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 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.)

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

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:

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

**yellow** \hspace{1cm} **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. 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 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 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.

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.
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 (aq) and smaller amount of In\(^{-}\) (aq). In order to measure the amount of HIn (aq) and In\(^{-}\) (aq) very precisely, you will convert the spectrophotometer readings you take in the lab to the molar concentration of HIn (aq) and In\(^{-}\) (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. 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. You will be given one calibration curve for HIn (aq) and another for In\(^{-}\) (aq).

After you determine the concentration of HIn (aq) and In\(^{-}\) (aq) using the spectrophotometer and calibration curves, you will measure the pH of your solutions with a pH meter. pH is a measure of the amount of hydronium ion, H\(_3\)O\(^{+}\) (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\(_3\)O\(^{+}\) (aq) in your colored solutions, you can use that information to describe the solutions numerically by calculating the equilibrium constant, \(K_{eq}\), for 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:

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

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

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

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

Once you have calculated the \(K_{eq}\) 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 textbook (e.g., Brown/LeMay/Bursten p. 219 or 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.
   a) What is the hydronium ion concentration, \([H_3O^+]\), of this solution?
   b) The solution has absorbance 0.336 at 430nm, which is the \( \lambda_{\text{max}} \) for HIn (aq). Use the HIn (aq) calibration curve to find [HIn] for the solution.
   c) The solution has absorbance 0.143 at 590nm, which is the \( \lambda_{\text{max}} \) for In⁻ (aq). Use the In⁻ (aq) calibration curve to find [In⁻] for the solution.
   d) Using your answers to parts a, b, and c, calculate \( K_{eq} \) for this solution.
   e) 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 cresol 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 – one wavelength is the absorbance maximum (λ_{max}) for the for acid (HIn) form of the indicator and the other wavelength is the absorbance maximum for the for base (In⁻) form of the indicator. After doing this you will adjust the solution you made to a different pH, and record its %transmittance at λ_{max} for HIn and λ_{max} for In⁻. You will repeat this three 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 pH and λ_{max} 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>Procedure and Calculations Flow Chart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Make a solution and adjust it to a different pH value for each trial</td>
</tr>
<tr>
<td>Trial 1</td>
</tr>
<tr>
<td>Trial 2</td>
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<tr>
<td>Trial 3</td>
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<tr>
<td>Trial 4</td>
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<tr>
<td>Trial 5</td>
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<tr>
<td>Measure pH</td>
</tr>
<tr>
<td>Measure absorbance at In⁻ peak</td>
</tr>
<tr>
<td>Measure absorbance at HIn peak</td>
</tr>
<tr>
<td>Calculate [H_{3}O⁺]</td>
</tr>
<tr>
<td>Use calibration curve to calculate [In⁻]</td>
</tr>
<tr>
<td>Use calibration curve to calculate [HIn]</td>
</tr>
<tr>
<td>Calculate K_{eq}</td>
</tr>
</tbody>
</table>

**Table 1 -- pH and wavelength settings for different indicators**

<table>
<thead>
<tr>
<th>Assigned indicator</th>
<th>pH values (adjust one trial to each pH value)</th>
<th>Absorbance maximum (λ_{max}) for HIn</th>
<th>Absorbance maximum (λ_{max}) for In⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromophenol blue</td>
<td>3.0, 3.4, 3.7, 4.0, 4.4</td>
<td>430</td>
<td>590</td>
</tr>
<tr>
<td>Cresol red</td>
<td>7.4, 7.7, 8.0, 8.3, 8.7</td>
<td>430</td>
<td>570</td>
</tr>
</tbody>
</table>

Making the acid-base indicator solution

1. Add 8.00 mL of the acid-base indicator you have been assigned to a 250 mL volumetric flask.
2. Empty a pH buffer capsule into a 400 mL beaker (use an orange pH 4 capsule)

***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.
if you are assigned to study bromphenol blue and a pH 8 capsule if you are assigned to study cresol red). Add about 100 mL deionized water. Stir to dissolve and then pour the solution into your flask.

3. Fill the flask to the line with deionized water. Invert the flask repeatedly until the solution is homogeneous.

**Calibrating the spectrophotometer**

1. Turn on the spectrophotometer.
2. Fill a cuvet full of colorless pH 4 or pH 7 buffer solution. This cuvet represents your blank.
3. Set the wavelength on the spectrophotometer to the wavelength you are using to measure your sample. Insert the blank into the sample chamber and close the lid.
4. Turn the zero (right-hand) knob until the instrument reads **100.0 on the transmittance scale**.

**Calibrating the pH meter**

1. Turn on the pH meter.
2. Make sure the electrode is immersed in pH 7 buffer solution.
3. Press “Cal/Meas” and wait for the pH meter to read “ready”.
4. Press “Enter”.
5. Remove the electrode from the pH 7 buffer. Rinse the electrode with deionized water from a wash bottle over a waste beaker.
6. Immerse the electrode in a small beaker of pH 4 buffer if you are studying bromphenol blue or pH 10 buffer if you are studying cresol red.
7. Wait for the pH meter to read “ready”.
8. Press “Enter”
9. Press “Cal/Meas”. The pH meter is now ready to use.

**Adjusting the pH of the solution**

1. Obtain a clean 100 mL beaker and pour about 60 mL of your solution from the volumetric flask into the beaker.
2. Add a small magnetic stir bar to the beaker and place it on the magnetic stir plate. Turn on the stir plate (medium speed).
3. Immerse the pH electrode in the solution in the beaker on the stir plate and wait for the pH reading to stabilize.
4. Add 1M HCl or 1M NaOH solution, depending on whether you need to make the solution more acidic or more basic, to the beaker very slowly, drop-by-drop with a disposable pipet. Stop adding acid when the pH is ± 0.1 units of the desired pH (see Table 1 for desired pH values).
5. Wait for the pH reading to stabilize. Then record the exact pH in your data table.

Reading the % transmittance of the solution at \( \lambda_{\text{max}} \) for HIn and \( \lambda_{\text{max}} \) for In\textsuperscript{2+}.

1. Using a clean disposable pipet, fill a cuvet about two-thirds full of the solution in your 100mL beaker.
2. 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.
3. Recalibrate the spectrophotometer (see calibrating the spectrophotometer above) to the second wavelength you need to measure at.
4. Save the cuvet containing your solution in a test tube rack.

**Data Table**

| Assigned indicator : |  |

<table>
<thead>
<tr>
<th>EQUIPMENT TO USE:</th>
<th>Your eyes</th>
<th>pH meter</th>
<th>Spectrophotometer at 430</th>
<th>Spectrophotometer at 570 (cresol red) or 590 (brom. blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial</td>
<td>Color</td>
<td>pH</td>
<td>% Transmittance at ( \lambda_{\text{max}} ) for HIn</td>
<td>% Transmittance at ( \lambda_{\text{max}} ) for In\textsuperscript{2+}</td>
</tr>
<tr>
<td>1</td>
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</tbody>
</table>
**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 = \(2 - \log (\% \text{ transmittance})\)

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

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

\[
K_{eq} = \frac{\text{Determine [In}^-\text{] from absorbance value at In}^-\text{ peak}}{\text{Determine [H}_3\text{O}^+\text{] from pH}} \div \frac{\text{Determine [HIn] from absorbance value at HIn peak}}{\text{Determine [HIn] from absorbance value at HIn peak}}
\]

<table>
<thead>
<tr>
<th>Trial</th>
<th>Absorbance at (\lambda)\text{max for HIn}</th>
<th>Absorbance at (\lambda)\text{max for In}^-</th>
<th>[H(_3)O(^+)]</th>
<th>[HIn]</th>
<th>[In(^-)]</th>
<th>(K_{eq})</th>
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</thead>
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<td>1</td>
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</tr>
</tbody>
</table>
**Literature comparison**

1. Calculate your mean value of \( K_{eq} \) by averaging the \( K_{eq} \) values for your five solutions.

2. The literature value of \( K_{eq} \) for bromophenol blue is \( 1.15 \times 10^{-4} \). The literature value of \( K_{eq} \) for cresol red is \( 6.31 \times 10^{-9} \). Calculate the percent error of your mean \( K_{eq} \) value using this equation:

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

**Post-lab questions and analysis**

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

2. Compare the \( K_{eq} \) values of cresol red and bromphenol blue.
   a. Which is larger?
   
   b. What do the different \( K_{eq} \) values tell you about these two reactions as compared to one another?
3. Why does the procedure call for groups who studied cresol red to adjust the pH of their solutions to different values (7.4, 7.7, 8.0, 8.3, 8.7) than the groups who studied bromphenol blue (3.0, 3.4, 3.7, 4.0, and 4.4)?

4. Compare these two solutions in terms of pH:
   - Cresol 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 AB, A₂B, or AB₂.

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

**Data set**

<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 × 10⁻¹</td>
<td>9.56 × 10⁻¹</td>
<td>3.56 × 10⁻³</td>
</tr>
<tr>
<td>3.24 × 10⁻³</td>
<td>6.50 × 10⁻³</td>
<td>1.84 × 10⁻⁷</td>
</tr>
<tr>
<td>5.25 × 10⁻¹</td>
<td>5.63 × 10⁻¹</td>
<td>4.86 × 10⁻²</td>
</tr>
<tr>
<td>5.60 × 10⁻⁴</td>
<td>1.20 × 10⁻⁰</td>
<td>1.18 × 10⁻¹⁴</td>
</tr>
<tr>
<td>5.2 × 10⁻⁵</td>
<td>1.83 × 10⁻¹</td>
<td>1.55 × 10⁻⁶</td>
</tr>
</tbody>
</table>

If the formula of the complex is 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 AB₂ then \( K_{eq} = \frac{[AB_2]}{[A^{2+}] \cdot [B^-]^2} \).

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

6. In this experiment, we made a significant assumption which introduces error in our calculations. We assumed that the absorbance of In⁻ is zero at the wavelength where HIn has maximum absorbance. That is, at \( \lambda_{max} \) for HIn, we assumed the absorbance is solely due to HIn. Likewise, we assumed that the absorbance of the conjugate base In⁻ is zero at the maximum absorption wavelength for HIn.

Look at the graph of the absorbance spectrum for the acid (HIn) and conjugate base (In⁻) forms of bromphenol blue below. What is the absorbance of In⁻ at the point where HIn has its \( \lambda_{max} \)? What is the absorbance of HIn at the \( \lambda_{max} \) of In⁻? Are the assumptions in the experiment valid for bromphenol blue?

Now look at the graph of the absorbance spectrum for the HIn and In⁻ forms of cresol red below. What is the absorbance of In⁻ at the \( \lambda_{max} \) of HIn? What is the absorbance of HIn at the \( \lambda_{max} \) of In⁻? Are the assumptions in the experiment valid for cresol red?
For which indicator, cresol red or bromphenol blue, do the assumptions introduce a larger experimental error? Explain your choice.

To which calculation -- that of [HIn] or of [In^-] -- does this error contribute the most? Would that cause the \( K_{eq} \) calculated to be higher or lower than it should be? Would the error introduced in calculating \( K_{eq} \) be greatest at higher or lower pH? Explain your reasoning.