**Determination of the Equilibrium Constant**

**Introduction**
Indicators are substances whose solutions change color due to changes in pH. They are usually weak acids or bases, but their conjugate base and acid forms have different colors due to differences in their absorption spectra. Indicators are typically weak acids or bases with complicated structures. For simplicity, we represent a general indicator by the formula $\text{HIn}^-$, and its ionization in a solution by the equilibrium,

$$\text{HIn}^- + \text{H}_2\text{O} = \text{H}_3\text{O}^+ + \text{In}^{2-},$$

and define the equilibrium constant as $K_{ai}$,

$$K_{ai} = \frac{[\text{H}_3\text{O}^+][\text{In}^{2-}]}{[\text{HIn}^-]}$$

In this experiment we will determine the equilibrium constant ($K_{eq}$) for the indicator Bromothymol blue using a spectrophotometer and a pH meter. Keep in mind that Bromothymol blue is blue when in the basic form ($\text{In}^{2-}$) and yellow when in the acidic form ($\text{HIn}^-$).

**Pre-lab Questions**
1. What is the initial concentration of bromothymol blue in the stock solution and each of the prepared solutions (C1 – C6 and S1 – S5)? Remember to use the $\text{M}_1\text{V}_1=\text{M}_2\text{V}_2$ equation.
2. What color do you expect each solution C1 – C6 to be? S1? S5?
3. If the solution reads pH 7.66, what is the $[\text{H}_3\text{O}^+]_{eq}$?

**Purpose**
Write an appropriate purpose for this lab.

**Materials and Methods**
Write an appropriate list for this experiment. Sketch any items that are new to you.

**Procedure**
**Part 1 – Tuning the Instrument Wavelength**
At some wavelengths bromothymol blue will absorb light intensely while at others it will be nearly completely transparent. Our goal is to tune the instrument to the wavelength that will give us the best signal. This will be accomplished by testing our calibration solutions at 20 nm intervals and selecting the wavelength of minimum transmittance (maximum light absorbed) for both the basic blue form and acidic yellow form. By using strongly acidic or basic solutions, we can shift the equilibrium nearly completely toward the basic or acidic forms of the indicator.
(1) Obtain a spectrophotometer and turn it on. Be sure to allow a few minutes for the instrument to “warm up”.

(2) Prepare 6 calibration solutions in medium test tubes using 1 and 5 ml graduated pipettes.

<table>
<thead>
<tr>
<th>Calibration Solution</th>
<th>0.00025M Bromothymol Blue (mL)</th>
<th>0.010 M HCl (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>1.00</td>
<td>4.00</td>
</tr>
<tr>
<td>C2</td>
<td>0.70</td>
<td>4.30</td>
</tr>
<tr>
<td>C3</td>
<td>0.50</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>0.00025M Bromothymol Blue (mL)</td>
<td>0.010 M NaOH (mL)</td>
</tr>
<tr>
<td>C4</td>
<td>1.00</td>
<td>4.00</td>
</tr>
<tr>
<td>C5</td>
<td>0.70</td>
<td>4.30</td>
</tr>
<tr>
<td>C6</td>
<td>0.50</td>
<td>4.50</td>
</tr>
</tbody>
</table>

(3) Make sure that the filter on the bottom left of the spectrophotometer is set to the proper range and adjust the wavelength knob for 400 nm.

(4) **With no tube in the instrument and the lid closed** adjust the amplifier knob (front left) until the instrument displays 0.0% transmittance.

(5) Place a “blank” tube with distilled water in the sample holder and close the lid. Adjust 100% transmittance knob (front right) until the instrument displays 100.0%

(6) **Without touching any knobs** remove the blank and place the tube containing solution **C1** in the instrument (C2 and C3 will be measured in Part 2). Record the % transmittance.

(7) Increase the wavelength by 20 nm. Repeat steps 5 and 6 by reinserting the “blank” and adjusting the right knob (but not the left) to tune the instrument to 100.0% transmittance. **Without touching any knobs** record % transmittance for solution **C1**.

(8) Proceed in this fashion in 20 nm intervals until 500 nm is reached and note the wavelength of minimum % transmittance for **HIn**.

(9) Adjust the wavelength knob for 550 nm.

(10) **With no tube in the instrument and the lid closed** adjust the amplifier knob (front left) until the instrument displays 0.0% transmittance.

(11) Place a “blank” tube with distilled water in the sample holder and close the lid. Adjust 100% transmittance knob (front right) until the instrument displays 100.0%

(12) **Without touching any knobs** remove the blank and place the tube containing solution **C4** in the instrument (C5 and C6 will be measured in Part 2). Record the % transmittance.

(13) Proceed as you did in steps 5 and 6, measure solution **C4** every 20 nm until you reach 650 nm. **Remember to switch the filter on the bottom left of the instrument when you go from 590 nm to 610 nm**. Note the wavelength of minimum % transmittance for **In**.  

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Part 2 – Determining the Relationship between Absorbance and Concentration

There are two common methods by which to measure the interaction of light with a sample. Our measurements were taken in % transmittance, which is a measure of the amount of light to pass through the sample. Absorbance \((A)\) is a measure of the amount of light absorbed by the sample rather than transmitted through. The below equation allows you to convert from % transmittance to absorbance.

\[
A = -\log\left(\frac{\% \text{trans}}{100}\right)
\]

Beer’s law states that absorbance \((A)\) is directly proportional to concentration in molarity. Let’s simplify Beer’s law to the below equation where \(n\) is a constant (a combination of the distance the light travels through the solution and how intensely the chemical absorbs light) and \(c\) is molarity.

\[A = nc\]

The value of \(n\) varies from one chemical to another so we will actually have two different values of \(n\) in this lab. The value of \(n\) for \(\text{HIn}^-\) shall be referred to as \(n_y\) and \(n\) for \(\text{In}^{2-}\) shall be referred to as \(n_b\) where the subscripts refer to the colors of those species in solution. Measuring \(n\) will allows us to determine the relationship between concentration and absorbance. Remember that each \(n\) will only be accurate at the wavelength determined in Part 1.

\[
A = n_y [\text{HIn}^-] \quad A = n_b [\text{In}^{2-}]
\]

(14) Tune the instrument to the wavelength of minimum transmittance for \(\text{HIn}^-\) determined in Part 1. Be sure the filter is set to the proper range.
(15) **With no tube in the instrument and the lid closed** adjust the amplifier knob (front left) until the instrument displays 0.0% transmittance.
(16) Place a “blank” tube with distilled water in the sample holder and close the lid. Adjust 100% transmittance knob (front right) until the instrument displays 100.0%.
(17) **Without touching any knobs** remove the blank and place the tubes containing solutions C1 – C3 in the instrument. Record the % transmittances.
(18) Tune the instrument to the wavelength of minimum transmittance for \(\text{In}^{2-}\) determined in Part 1. Be sure the filter is set to the proper range.
Part 3 – Testing the Behavior of Bromothymol Blue at different pH’s

(20) Prepare 5 standard solutions in medium test tubes using 1 and 5 ml graduated pipettes.

<table>
<thead>
<tr>
<th>Solution</th>
<th>0.00025M Bromothymol blue (mL)</th>
<th>0.10M K2HPO4 (mL)</th>
<th>0.10M KH2PO4 (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>1.00</td>
<td>4.00</td>
<td>0.00</td>
</tr>
<tr>
<td>S2</td>
<td>1.00</td>
<td>3.00</td>
<td>1.00</td>
</tr>
<tr>
<td>S3</td>
<td>1.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>S4</td>
<td>1.00</td>
<td>1.00</td>
<td>3.00</td>
</tr>
<tr>
<td>S5</td>
<td>1.00</td>
<td>0.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

(21) Tune the instrument to the wavelength of minimum transmittance for $\text{HIn}^-$ determined in Part 1. Be sure the filter is set to the proper range.

(22) **With no tube in the instrument and the lid closed** adjust the amplifier knob (front left) until the instrument displays 0.0% transmittance.

(23) Place a “blank” tube with distilled water in the sample holder and close the lid. Adjust 100% transmittance knob (front right) until the instrument displays 100.0%

(24) **Without touching any knobs** remove the blank and place the tubes containing solutions S1 – S5 in the instrument. Record the % transmittances.

(25) Tune the instrument to the wavelength of minimum transmittance for $\text{In}^{2-}$ determined in Part 1. Be sure the filter is set to the proper range.

(26) Repeat steps 22 – 24 with solutions S1 – S5.

(27) Use the pH meter to record the pH of solutions 1 – 5. You may need to transfer the solution to a 50 mL beak or large test tube in order to immerse the pH probe.

**Data Tables**

Prepare appropriate tables for the data you are collecting. You will need to have a data and analysis section for each part, since you need the results from the previous part’s analysis to do the next part. It will make the most sense in your notebook if you write the procedure and set up the data tables and analysis section for each section before writing the next section.

**Analysis**

- Wavelength of maximum absorbance (minimum %T) for acidic and for basic forms of bromothymol blue.
- Use Excel to graph a calibration curve, $A$ vs. bromothymol blue concentration for both the yellow form and the blue form. There should be one plot for C1 – C3 and another plot for C4 – C6. Use the equation of the line in Excel to obtain $n_y$ and $n_b$.
- Calculate $K_{ai}$ for each solution S1 – S5. Provide one set of calculations showing work. Average the five values of $K_{ai}$ and calculate standard deviation for $K_{ai}$.
- Assess the amount of error in this experiment. Does $[\text{In}^{2-}] + [\text{HIn}^-] = \text{initial bromothymol blue concentration}$ according to your calculations as it should for solutions S1 – S5?
Conclusion

Post-lab Questions
1. Why is Part 1 necessary to achieving good results?
2. Why were multiple concentrations used in Part 2 and why were multiple pH’s used in part 3.