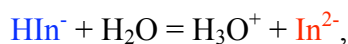


Determination of the Equilibrium Constant

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 HIn^- , and its ionization in a solution by the equilibrium,



and define the equilibrium constant as K_{ai} ,

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

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 (In^{2-}) and yellow when in the acidic form (HIn^-).

Pre-lab Questions

1. What is the initial concentration of bromothymol blue in solution 1 and each of the solutions (C1 – C6 and S1 – S5)? Remember to use the $M_1V_1=M_2V_2$ equation.
2. What color do you expect solution 1 to be? Solution 5?
3. If the solution reads pH 7.66, what is the $[\text{H}_3\text{O}^+]_{\text{eq}}$?

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.

Calibration Solution	0.00025M Bromothymol Blue (mL)	0.010 M HCl (mL)
C1	1.00	4.00
C2	0.70	4.30
C3	0.50	4.50
Calibration Solution	0.00025M Bromothymol Blue (mL)	0.010 M NaOH (mL)
C4	1.00	4.00
C5	0.70	4.30
C6	0.50	4.50

- (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^{2-} .

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.

$$A = -\log\left(\frac{\%trans}{100}\right)$$

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 HIn^- shall be referred to as n_y and n for In^{2-} shall be referred to as n_b where the subscripts refer to the colors of those species in solution. Measuring n will allow us to determine the relationship between concentration and absorbance. Remember that each n will only be accurate at the wavelength determined in Part 1.

$$A = n_y [\text{HIn}^-]$$

$$A = n_b [\text{In}^{2-}]$$

- (14) Tune the instrument to the wavelength of minimum transmittance for 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 In^{2-} determined in Part 1. Be sure the filter is set to the proper range.
- (19) Repeat steps 15 – 17 with solutions C4 – C6.

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**.

Solution	0.00025M Bromothymol blue (mL)	0.10M K ₂ HPO ₄ (mL)	0.10M KH ₂ PO ₄ (mL)
S1	1.00	4.00	0.00
S2	1.00	3.00	1.00
S3	1.00	2.00	2.00
S4	1.00	1.00	3.00
S5	1.00	0.00	4.00

- (21) Tune the instrument to the wavelength of minimum transmittance for 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 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.

Calculations

- 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.
- Calculate an average n_y , n_b and K_{ai} .

Post-lab Assignment

- Present all data neatly and creatively in data tables.
- Provide one set of sample calculations.
- Assess the amount of error in this experiment. How much did your values for n_y , n_b and K_{ai} vary? Does $[\text{In}^{2-}] + [\text{HIn}^-] = \text{initial bromothymol blue concentration}$ according to your calculations as it should for solutions S1 – S5?
 1. Explain why Part 1 is necessary to achieving good results.
 2. Explain why multiple concentrations were used in Part 2 and why multiple pH's were used in part 3.