

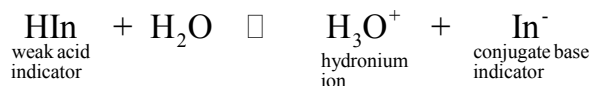
## Experiment 6

### Determination of the Equilibrium Constant for Bromocresol Green

#### Goals

#### Discussion

Acid-base indicators are often used to demonstrate the end-point of an acid-base reaction. Acid-base indicators are weak acids that dissociate into a hydronium ion ( $\text{H}_3\text{O}^+$ ) and an anion ( $\text{In}^-$ ). This dissociation can be represented through the following equation:



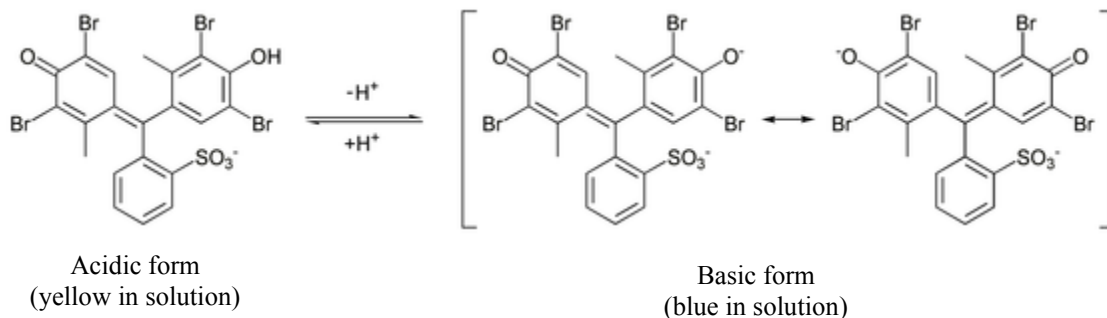
In order for a compound to be a useful indicator, the acidic form ( $\text{HIn}$ ) and the basic form ( $\text{In}^-$ ) of the indicator should vary greatly in color. Since the equilibrium in acid solution favors the formation of  $\text{HIn}$ , this species is called the acidic form of the indicator. Likewise, the  $\text{In}^-$  form of the compound the basic form since it is favored in basic solutions. An equilibrium mixture of the indicator will be colored according to the relative concentration of each form of the indicator. The position of the equilibrium and, therefore, the relative concentration of the two forms of the indicator will depend on the  $\text{H}_3\text{O}^+$  concentration,  $[\text{H}_3\text{O}^+]$  or  $[\text{H}^+]$ .

The study of the absorption curves of an indicator at different pH values provides an excellent method for studying the color changes occurring in acid-base indicators. It also allows us to determine the equilibrium constant of the indicator, which in terms of the acid form and base form is

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

**In this experiment, we will determine the equilibrium constant of bromocresol green (BCG).** BCG is an indicator in which the acidic form is yellow and the basic form is blue. Bromocresol green is purchased as a solid sodium salt and behaves as a simple monoprotic acid. When dissolved in water the conjugate pair (acid and base forms) display different absorption spectra since they are different colors.

#### Structure of Bromocresol Green ( $\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$ , MM = 698 g/mol)



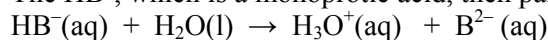
For simplicity we'll use the following symbols to represent bromocresol green:

- H<sub>2</sub>B The fully protonated form of the indicator
- HB<sup>-</sup> The deprotonated form of the indicator
- NaHB The sodium salt of the deprotonated form of the indicator
- B<sup>2-</sup> The fully deprotonated form of the indicator

The sodium salt of bromocresol green ionizes completely in water:



The HB<sup>-</sup>, which is a monoprotic acid, then partially dissociates to give B<sup>2-</sup>:



Writing the dissociation without water, we have



The equilibrium constant for this reaction is

$$K = \frac{[\text{H}^+][\text{B}^{2-}]}{[\text{HB}^-]}$$

The HB<sup>-</sup> is the acidic form and is yellow in solution. The B<sup>2-</sup> is the basic form and is blue in solution.

Taking logarithms of the above equation gives

$$\log K = \log[\text{H}^+] + \log \frac{[\text{B}^{2-}]}{[\text{HB}^-]}$$

$$\log \frac{[\text{B}^{2-}]}{[\text{HB}^-]} = \text{pH} + \log K$$

$y = m x + b$

where we have rearranged and noted that  $\text{pH} = -\log [\text{H}^+]$ .

This is a linear equation. A plot of  $\log \frac{[\text{B}^{2-}]}{[\text{HB}^-]}$  versus pH should yield a straight line with a slope of 1 ( $m = 1$ ) and an intercept equal to  $\log K$ , where  $K$  is a concentration equilibrium constant.

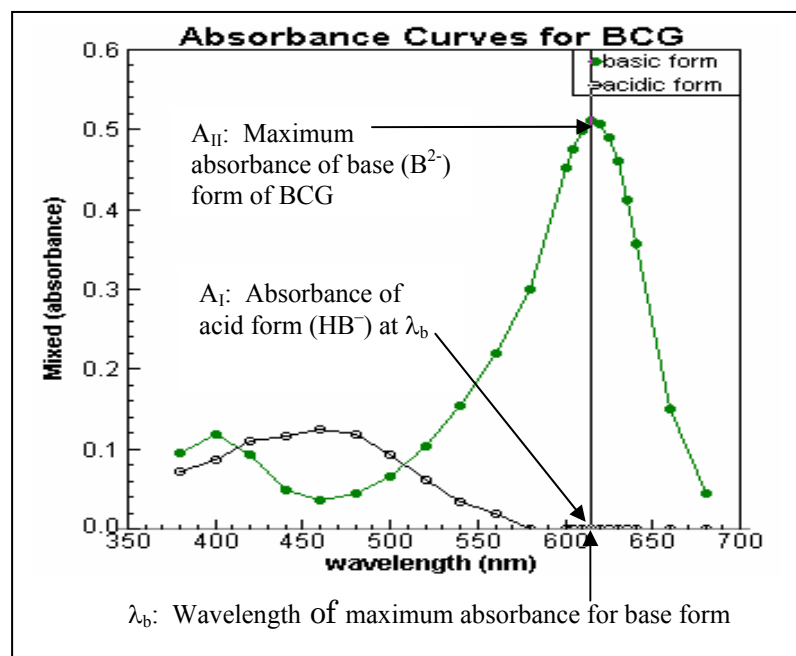
### Absorbance and Spectrophotometry

Solutions that possess colors absorb visible light energy of specific wavelengths. Recall that a red solution appears red because it absorbs much of the blue-green part of the spectrum (complementary colors). Measurements of the amount of light absorbed by a substance at each wavelength (color) can be graphed giving an "absorption curve." The shape of this curve depends almost entirely on the electronic structure of the substance and is almost unique for each substance. Thus the curve serves as an aid to identification and, with the aid of modern theory, a clue to the structure of a substance.

At a given wavelength the amount of light absorbed by a solution is proportional to its molar concentration, thus providing a widely used method of concentration analysis. The Beer-Lambert Law states that  $A = \epsilon lc$ , where  $A$  = absorbance,  $\epsilon$  = a constant characteristic of the absorbing molecule,  $l$  = path length,  $c$  = concentration. In our case,  $\epsilon$  and  $l$  are constant (known absorbing substance and a path length determined by the width of the cuvette). Thus the absorbance is proportional to the concentration in this experiment.

A spectrophotometer is an instrument used for measuring the amount of absorption at different wavelengths. Many such instruments are now commercially available. Some are designed to operate in the region of visible light, some in infra-red regions, some in ultra-violet and still others in several of these energy bands. You will use either a Spectronic 301 Spectrophotometer or a Genesys 20 Spectrophotometer to measure absorbance in this experiment. Both instruments are designed for use in the visible portion of the spectrum (400 to 700 nanometers, nm). Instructions for operating the Spectronic 301 are on the last page of this experiment. Operation of the Genesys 20 is similar.

### Determining the Equilibrium Constant



If the acid ( $HB^-$ ) and base ( $B^{2-}$ ) forms of the indicator are both colored, the ratio  $\frac{[B^{2-}]}{[HB^-]}$  can be determined by measuring the absorption of light at the wavelength at which one of the forms absorbs a lot and the other form absorbs a little.

Plots of the absorption of light of various wavelengths ( $\lambda$ ) versus wavelength for BCG in its basic form and in its acidic form are shown. Such graphs are called absorption spectra.

The graph shows that the basic form has maximum absorbance ( $A_{II}$ ) at  $\lambda_b$ . The absorbance of the acid form ( $A_I$ ) is small at  $\lambda_b$ .

If we start with a solution of the pure basic form ( $B^{2-}$ ) and add an acid to the solution (for example, acetic acid), then some of the basic form will be converted to the acid form ( $HB^-$ ). Then the absorbance at  $\lambda_b$  will drop because the acidic form absorbs almost no light at  $\lambda_b$ . This change is visible in that the solution will change from blue to yellow.

An absorbance measurement with some acid form and some base form is noted by  $A_x$ . In fact, all absorbance values along the vertical line drawn at  $\lambda_b$  in the graph are  $A_x$  values. These points all lie between  $A_{II}$  and  $A_I$ .

Referring back to the equation,  $\log \frac{[B^{2-}]}{[HB^{-}]} = pH + \log K$ , we see that in order to calculate the equilibrium constant,  $K$ , different ratios of  $\frac{[B^{2-}]}{[HB^{-}]}$  must be found for different pH values. When these are plotted, a straight line with slope of one and y-intercept of  $\log K$  is obtained. A sample graph is on page F-9. The relationship between the absorbance data at  $\lambda_b$  and the ratio  $\frac{[B^{2-}]}{[HB^{-}]}$  is found from the following equation:

$$\frac{[B^{2-}]}{[HB^{-}]} = \frac{A_x - A_I}{A_{II} - A_x} \text{ where}$$

$A_I$  = absorbance of the solution when all indicator is in the acidic form (pure  $HIn$ ).

$A_{II}$  = absorbance of the solution when all indicator is in the basic form (pure  $In^{-}$ ).

$A_x$  = absorbance of the solution when both some acidic and some basic form are present

Equipment: Spectrophotometer, two cuvettes, Pipette Pump, 3–250 mL beakers, 100 mL graduated cylinder, 2 mL pipet, 10 mL graduated cylinder, Kimwipes, 3 1/2 inch PC formatted diskette or USB flash drive  
Chemicals: 1.00 M acetic acid,  $3.00 \times 10^{-4}$  M bromocresol green, 0.200 M sodium acetate, cuvette containing acidic form of BCG obtained from your instructor.

Students will work in groups as assigned by the instructor. **Use eye protection for this experiment.**

- 1. BROMOCRESOL GREEN SOLUTION:** Add 5.00 ml of  $3 \times 10^{-4}$  M bromocresol green solution and 5.00 mL of 0.200 M sodium acetate solution to a 100 mL graduated cylinder. Dilute to 100.0 mL with distilled water and pour quantitatively (this means all of the solution) into a clean, dry 250-mL beaker. The sodium acetate molarity of this solution is 0.0100 M.
- 2. 0.250 M ACETIC ACID SOLUTION:** Rinse the graduated cylinder with distilled water, add 25.0 mL of the 1.00 M acetic acid and dilute to 100.0 mL with distilled water. Pour into a clean, dry 250-mL beaker. This is the solution you will add to the basic form of the bromocresol green to get in-between absorbance values. The molarity of this solution is 0.250 M acetic acid.
- 3. BLANK SOLUTION:** Add 5 mL of 1.00 M acetic acid and 5 mL of 0.200 M sodium acetate to a 100 mL graduated cylinder and dilute with distilled water to 100 mL.
4. Measure the absorption spectrum of the bromocresol solution from 380-680 nm at 20 nm intervals. Record these measurements in the Data Sheet.
5. Review the absorbance data and locate the largest value of the absorbance. Note what wavelength this absorbance corresponds to. Now take absorbance readings at 5 nm intervals from 20 nm below to 20 nm above this wavelength. For example, if the wavelength at maximum absorbance in the 20 Interval table is 620 nm, take readings at 5 nm intervals from 600 nm to 640 nm. However, don't duplicate readings—in this case don't take readings at 600 nm, 620 nm, and 640 nm.
6. After you are finished taking the spectrum, pour the bromocresol green that is in the cuvette back into the 250 ml beaker, taking care not to lose any because known volumes are needed for the calculations.
7. The instructor will supply a cuvette containing a bromocresol green solution that has excess acid (HCl) added to it. Measure the absorption spectrum of this solution at 20 nm intervals and record the values in the Data Sheet. There is no need to make measurements every 5 nm for the acid form of the indicator.
8. Return the cuvette containing the yellow form of BCG to your instructor.
9. From the data taken for the basic and acidic forms, decide which wavelength is suitable for measuring  $A_x$  values and set the spectrophotometer at this wavelength. The suitable wavelength,  $\lambda_b$ , is the wavelength in which the basic form has a maximum absorbance and the acidic form has a very small absorbance.
10. Add to the 250-mL beaker of bromocresol green solution precisely 2.00 mL (quantitatively) of the 0.250 M acetic acid solution. Use a 2-mL pipet and a Pipette Pump. Mix well with a stirring rod, and measure the absorption at  $\lambda_b$ . Quantitatively pour the solution in the cuvette back into the 250-mL beaker. Repeat this procedure for an additional 2.00 mL increment of acetic acid.
11. Add another 2.00 mL of the 0.250 M acetic acid solution, mix well, and measure the absorption at  $\lambda_b$ . Again, pour the solution in the cuvette back into the beaker. Repeat this procedure with a fourth 2.00-mL increment of the 0.250 M acetic acid solution. There should now be a total of 8.00 mL of 0.250 M acetic acid solution added to the bromocresol green solution.

1. Open Graphical Analysis on the computer.
2. Enter the combined absorbance data for the acid form and base form. Sort the data by wavelength. Label this data column (y-axis) “basic form” and enter “absorbance” for the units. Label the x-axis “wavelength” with units of “nm.”
3. To graph the acidic form absorbances in Graphical Analysis, you will need to add a second column to the Data Table Window. Do this by selecting New Column in the Data menu and choosing Manually Entered. Label the acidic data “acidic form” and enter “absorbance” for the units. Choose different point protectors for each of the two absorbance curves. Put a legend on the graph by checking Legend in the Graph menu. This way you will be able to distinguish the curves.
4. You have now plotted two absorption spectra for bromocresol green—the blue basic form and the yellow acidic form. Also, in addition to  $A_I$  and  $A_{II}$ , you should have four precise measurements of the absorbance ( $A_x$ ) of the bromocresol green solution at four different pH values. All these measurements have been taken at  $\lambda_b$ , the wavelength where the absorbance of the basic form of the indicator is at a maximum and the absorbance of the acidic form is very low.

The other data necessary for determining  $K$  are the pH values for the 4 additions of 0.025 M acetic acid solution to the BCG solution. Since the resulting mixtures contain both acetic acid and sodium acetate, the mixtures are buffer solutions. The pH of these buffer solutions can easily be calculated from the Henderson-Hasselbalch equation. Since the volume is the same for both the  $\text{Ac}^-$  and the  $\text{HAc}$  solutions, we can use moles instead of molarity to find the pH.

$$\text{pH} = \text{pK} + \log \frac{[\text{Ac}^-]}{[\text{HAc}]}$$

$$\text{pH} = \text{pK} + \log \frac{(\text{mol Ac}^-)}{(\text{mol HAc})}$$

5. When  $A_x$  was measured the concentration of the bromocresol green was not constant because we added volumes of acetic acid solution. To correct for the effect of dilution we multiply each measured value by:

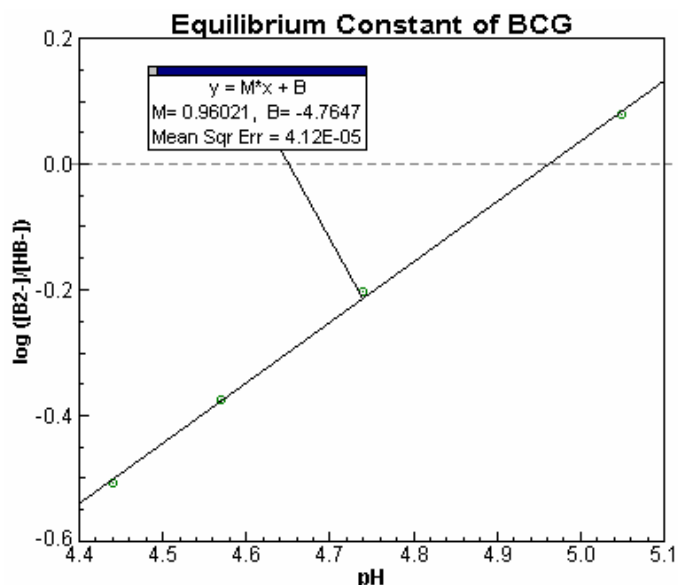
$$\text{Correction factor: } \left( \frac{100 \text{ mL} + V}{100 \text{ mL}} \right) \text{ where } V =$$

the milliliters of acetic acid solution added (either 2, 4, 6, or 8 mL).

The absorbance can be corrected for the effect of dilution by multiplying the correction factor by the measured absorbance:

$$A_{\text{corrected}} = A_{\text{measured}} \left( \frac{100 \text{ mL} + V}{100 \text{ mL}} \right)$$

6. You should already have a plot of both absorption curves on the same graph. Indicate the positions of the  $A_x$  values on the graph by drawing in a dot at the appropriate place. Clearly indicate on the graph the  $A_I$  and  $A_{II}$  values.



7. Complete the Data and Calculation Tables.

8. Plot the  $\log \frac{[B^{2-}]}{[HB^-]}$  versus pH using Vernier's Graphical Analysis. Fill out the Text Box, save your data, , and print the Whole Screen (data, graph and text box together). The y-intercept of the line, b, is the logarithm of the equilibrium constant for bromocresol green. A sample graph is shown below.

## Report

1. Hand in your original data tables, calculation tables, graphs, calculations and results.
2. Clearly indicate in your report the value of the equilibrium constant of BCG, K, and how you calculated it.

Answer the following question on a separate piece of paper:

3. When we calculate the pH of the solutions it's found by using the following equation:

$$\text{pH} = \text{pK} + \log \frac{(\text{mol Ac}^-)}{(\text{mol HAc})}$$

This assumes that the bromocresol green doesn't affect the overall pH. Why is this a good assumption?

### Operating Instructions for the Spectronic 301 Spectrophotometer

1. If the Spec 301 is not turned on, notify your instructor or the lab technician. Write the SPEC# of your instrument on your data sheet.
2. The Spec 301 should be in the ABSORBANCE mode. This is shown by a red light next to the letters ABS in the display. If the red light is next to any other mode, press the [SECOND FUNCTION] and [8(ABS)] keys.
3. The numbers in the display show the current wavelength and the absorbance value. For example, the display below shows readings of 685 nm and an absorbance of 0.536.

WAVELENGTH	DATA
685	0.536

4. Open the front cover of the Spec 301 and place a square plastic cuvette that contains the BLANK solution in the cuvette holder. Be sure that the cuvette is oriented so that the clear sides face front and back, and the outside surface is dry. Close the front cover.
5. Now set the desired wavelength: On the numeric pad, enter a three digit number for the wavelength (in nanometers). Then press [GO TO  $\lambda$ ], which is the up arrow key on the keypad. Your desired wavelength should now be shown, along with a previous absorbance reading (not the value you are looking for).
6. Press [AUTO ZERO] on the keypad. The DATA value should now read 0.000 absorbance.
7. Open the front cover, remove the BLANK cuvette, and insert the SAMPLE cuvette into the cuvette holder. Be sure that the cuvette is oriented so that the clear sides face front to back, and the outside surface is dry. Close the front cover.
8. Read the absorbance on the digital display. Enter this absorbance value in your data sheet.
9. Remove the SAMPLE cuvette. Insert the BLANK cuvette. Set the wavelength to the next desired value as described in step #5 above. Continue with these instructions for all the desired wavelengths.
10. If you are collecting data for an absorbance vs molarity curve at constant wavelength, then just insert various SAMPLE cuvettes and take absorbance readings. Changing wavelengths, and using a BLANK cuvette after the first reading in the series, are not necessary.

20 nm Intervals		
wavelength (nm)	absorbance of basic form	absorbance of acidic form
380		
400		
420		
440		
460		
480		
500		
520		
540		
560		
580		
600		
620		
640		
660		
680		

5 nm Intervals for basic form	
wavelength (nm)	absorbance (basic)

$\lambda_b =$  \_\_\_\_\_ nm. This is the wavelength having the largest absorbance of all the data you took for the basic form in the above tables.

Absorbance (all taken at $\lambda_b$ )	$A_x$ (2 mL)	$A_x$ (4 mL)	$A_x$ (6 mL)	$A_x$ (8 mL)
Measured				
Corrected*				

\*See page 6 for a discussion of calculating the corrected absorbances.

**Table 1: Moles of acid/base**

Volume of HAc solution added (mL)	M HAc (all entries the same)*	liters HAc solution added	mol HAc added	[NaAc] (all entries the same)**	Volume of NaAc solution (all entries the same) (L)	mol NaAc (all entries the same)
2.00 mL		0.00200				
4.00 mL		0.00400				
6.00 mL		0.00600				
8.00 mL		0.00800				

\* This is the molarity of the 0.250 M ACETIC ACID SOLUTION.

\*\* This is the molarity of NaAc in the BROMOCRESOL GREEN SOLUTION. See page 4.

\*\*\* This is the solution that also contains the BCG.

**Table 2: Moles of acid/base and pH**

Volume of HAc solution added (mL)	mol HAc (from above table)	mol Ac <sup>-</sup> (from above table)	$\frac{(\text{mol Ac}^-)}{(\text{mol HAc})}$	$\log[(\text{mol Ac}^-)/(\text{mol HAc})]$	pK <sub>a</sub> for HAc	pH
2.00 mL					4.74	
4.00 mL					4.74	
6.00 mL					4.74	
8.00 mL					4.74	

**Table 3: Equilibrium Constant K**

A <sub>I</sub>	A <sub>II</sub>	Corrected A <sub>x</sub>	A <sub>x</sub> - A <sub>I</sub>	A <sub>II</sub> - A <sub>x</sub>	$\frac{(A_x - A_I)/A_{II} - A_x}{[B^{2-}]/[HB^-]}$	$\log [B^{2-}]/[HB^-]$	pH (from above table)