# Spectrophotometric Determination of the pKa of Bromothymol Blue

INTRODUCTION

Acid-base indicators are compounds that are simply weak acids (or bases) that exhibit different colors depending on whether they are present in solution as their acidic form (HIn) or as their basic form (In⁻). As the pH of a solution containing the indicator changes, the equilibrium shown below will be driven either towards reactants (HIn), or products (In⁻) causing the solution color to change depending on the concentration of each form present. For example, in strongly acidic solution, most of the indicator will be present in the form HIn, causing the solution color to correspond to that of HIn. In strongly basic solutions, most of the indicator will be in the In⁻ form, resulting the color 2 dominating the solution. At intermediate pH values, the solution color will be a mix of color 1 and color 2, depending on the relative amounts of HIn and In⁻ present.

$$HIn \quad = \quad H^+ \quad + \quad In^- \tag{1}$$

Color 1   Color 2
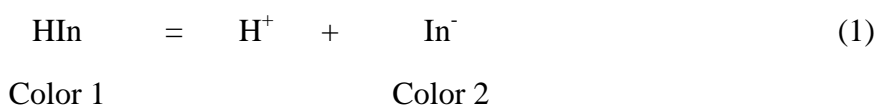
Quantitatively, the relationship between the pH (really the hydronium ion concentration) and the relative amounts of HIn and In⁻ in solution is described by the acid dissociation constant (K_a) for the indicator, as shown in Equation 2, where the square-bracketed terms represent the molar concentrations of each species in solution (M).

$$K_a = \frac{[H^+][In^-]}{[HIn]} \tag{2}$$

In practice, we typically measure the pH of a solution, rather than the [H⁺], therefore, it is useful to perform a few mathematical manipulations to the K_a expression to get things in terms of experimentally-determined quantities. We begin by taking the negative logarithm of both sides of the equation, resulting in the following:

$$-\log K_a = -\log[H^+] - \log\left(\frac{[In^-]}{[HIn]}\right) \tag{3}$$

That equation can be rewritten as

$$pK_a = pH - \log\left(\frac{[In^-]}{[HIn]}\right) \tag{4}$$

An examination of Equation 4 suggests that if we are able to monitor the relative concentrations of HIn and In⁻, it should be possible to determine the K_a for the indicator (or any weak acid for that matter).

While monitoring the pH of a solution using a glass electrode is fairly straightforward, determination of the relative concentrations of the two forms of the indicator requires a little more effort. The approach used in this experiment involves taking advantage of the fact that the acidic (HIn) and basic (In⁻) forms of the indicator absorb more strongly at different wavelengths.

Consider a generic indicator (HIn). At low pH the indicator is completely in the HIn form and the absorbance which is due to HIn is at a maximum (Figure 1A). Likewise, at high pH the indicator is completely in the In⁻ form and the absorbance which is due to In- is at a maximum (Figure 1B). At intermediate pH, the solution contains appreciable concentrations of both HIn and In⁻, and exhibits an absorbance spectrum containing contributions form both forms. As pH changes, the relative concentrations of each form will change in accordance with the K_a of the indicator, and the resulting absorbance of each form will also change (remember Beer's law?).

A   **B**   **C**
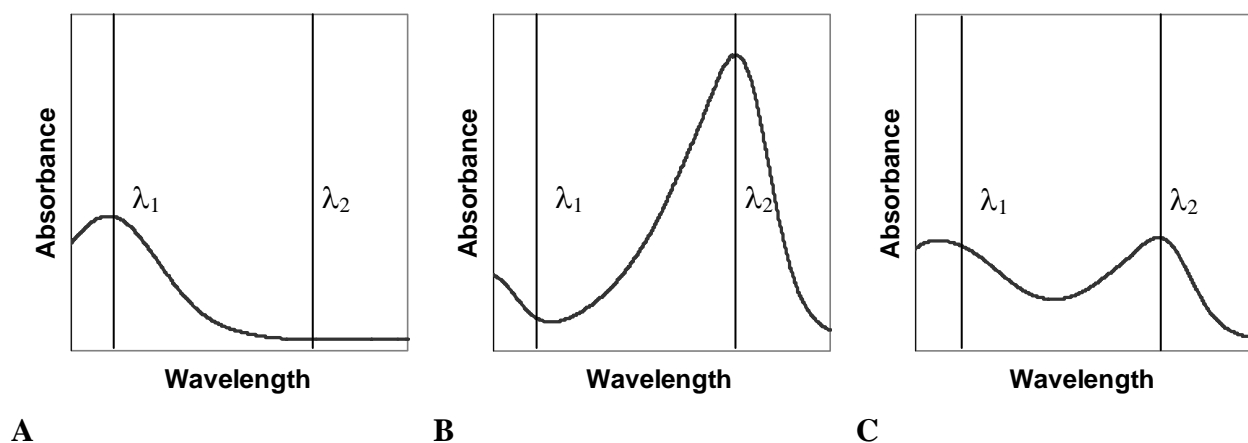
Figure 1. Example absorbance spectra of an acid base indicator in (A) acidic solution, (B) basic solution , and (C) solution of intermediate pH.

By monitoring the change in absorbance of each form as a function of solution pH, it is possible to extract a value for the K_a of the indicator. From equation 4, the solution pH will be equal to the pKa of the indicator when the concentrations of HIn and In⁻ in solution are equivalent. Experimentally, this corresponds to the pH where the absorbance of each form is half of its maximum absorbance. At this point, half of the initial number of moles of HIn have been converted to an equivalent number of moles of In⁻. As a result, the concentrations of each species are equal and the log term in Equation 4 becomes zero. Consequently, the pKa of an indicator corresponds to the pH of the solution at the inflection point in a plot of absorbance as a function of pH as shown in Figure 2. Note, however, that it is critical that the absorbance at each wavelength corresponds to the absorbance of only HIn at λ_1 and only In⁻ at λ_2.
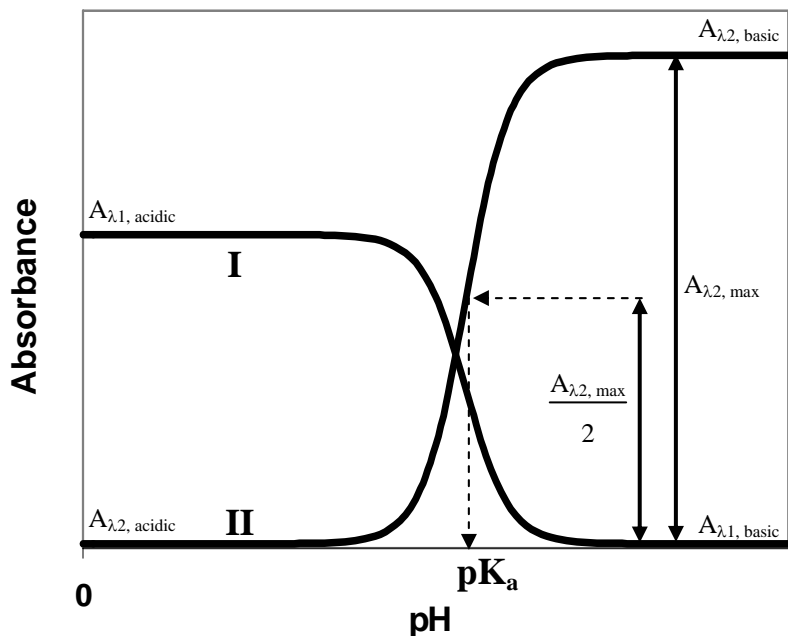
FIGURE 2. A plot of absorbance as a function of pH for an indicator. I- measurements which are made at a wavelength ($\lambda 1$) at which HIn absorbs radiation; II - measurements which are made at a wavelength ($\lambda 2$) at which In⁻ absorbs radiation.

Rearranging equation 4 results in the most common form of the Henderson-Hasselbach equation for the indicator. This expression provides an alternative way to determine the pKa of an indicator by plotting pH (y axis) as a function of log([In⁻]/[HIn]). As shown in Figure 3, the resulting straight line intersects the pH axis when pH equals pKa.

$$pH = pK_a + \log\left(\frac{[In^-]}{[HIn]}\right) \tag{5}$$
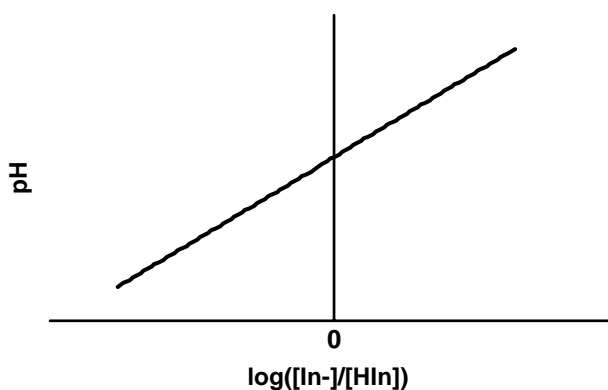
FIGURE 3. A plot of pH as a function of log([In⁻]/[HIn]). The pK$_a$ of the indicator corresponds to the intersection of the line with the pH axis.

It is possible to obtain the ratio [In-]/[HIn] to use for the plot from spectrophotometric measurements which are made at two wavelengths. The first wavelength (λ_1) is chosen where the acidic (HIn) but not the basic form (In⁻) of the indicator strongly absorbs radiation. The second wavelength (λ_2) is chosen where the basic but not the acid form strongly absorbs radiation. If Beer's law is obeyed, the absorbance at λ_1 and λ_2 are:

$$A_{\lambda 1} \quad = \quad \varepsilon_{(HIn,\lambda 1)} \bullet b \bullet [HIn] \tag{6}$$

$$A_{\lambda 2} \quad = \quad \varepsilon_{(In-,\lambda 2)} \bullet b \bullet [In^-] \tag{7}$$

where A is absorbance, ε is molar absorptivity and b is cell pathlength.

At any pH, the total concentration (C_T) of both forms of the indicator is constant and the sum of the individual concentrations of each species.

$$C_T \quad = \quad [HIn] + [In^-] \tag{8}$$

In solutions of low pH, essentially all of the indicator is in the acid form. Consequently, in highly acid solutions, $C_T = [HIn]$, and

$$A_{\lambda 1,acidic} \quad = \quad \varepsilon_{(HIn,\lambda 1)} \bullet b \bullet C_T \tag{9}$$

In highly basic solutions essentially all of the indicator is in the basic form and $C_T = [In^-]$ so that

$$A_{\lambda 2, basic} \quad = \quad \varepsilon_{(In-,\lambda 2)} \bullet b \bullet C_T \tag{10}$$

Taking a ratio of equations (6) to (9) and (7) to (10) provides expressions for the fraction of the indicator present in each form:

$$\frac{A_{\lambda 1}}{A_{\lambda 1,acidic}} = \frac{\varepsilon_{In^-} b [HIn]}{\varepsilon_{In^-} bC_T} = \frac{[HIn]}{C_T} \tag{11}$$

$$\frac{A_{\lambda 2}}{A_{\lambda 2,basic}} = \frac{\varepsilon_{In^-} b [In^-]}{\varepsilon_{In^-} bC_T} = \frac{[In^-]}{C_T} \tag{12}$$

The ratio [In-]/[HIn] at any pH can be obtained by dividing equation (11) by equation (12) to give:

$$\frac{[In^-]}{[HIn]} = \frac{A_{\lambda 2} / A_{\lambda 2,basic}}{A_{\lambda 1} / A_{\lambda 1,acidic}} = \frac{A_{\lambda 2} \bullet A_{\lambda 1,acidic}}{A_{\lambda 2,basic} \bullet A_{\lambda 1}} \tag{13}$$

The latter equation can be used to determine [In⁻]/[HIn] from absorbance data taken at any pH, as long as <u>the absorbances used in the equation are background corrected</u>. This correction accounts for contributions of the absorbance of other species in solution to the absorbance at λ_1 and λ_2.

For example, at λ_2 we assume that the measured absorbance is due only to the basic form of the indicator. In fact, the acidic form (or other species in solution) may absorb somewhat at λ_2, and conversely the basic form may absorb at λ_1. To correct for this you must subtract the minimum (or background) absorbance measured at λ_2 from each of the other measurements made at that wavelength. For example,

$$A_{\lambda2} = A_{\lambda2(measured)} - A_{\lambda2(min)}. \qquad\qquad (14)$$

The value $A_{\lambda2(min)}$ should come from the measurement made of your most acidic solution at λ_2.

In the experiment the pK_a of bromothymol blue (3',3"- dibromo-thymolsulfonephthalein) is determined by the two methods which have been discussed. At pH less than 6, the indicator is yellow and at pH greater than 7.6, the indicator is blue. At an intermediate pH, the blue and yellow combine to yield a green solution.

Reference

R. D. Braun, "Introduction to Chemical Analysis", McGraw-Hill, New York, 1982, pp. 197-199.

Prelaboratory assignment: The absorbance of a pH 6.85 bromothymol blue solution was 0.7333 at 430 nm and 0.569 at 555 nm. The minimum absorbance at 430 nm was 0.132, and the minimum absorbance at 555 nm was 0.021. The absorbance of a pH 1 solution of the same concentration of bromothymol blue was 1.097 at 430 nm and the absorbance of a pH 13 solution of the same concentration of bromothymol blue was 1.428 at 555 nm. Use the method described in the calculations section to calculate the ratio [In⁻]/[HIn] in the solution.

REAGENTS AND APPARATUS

- Cuvets
- 10-mL graduated cylinder
- pH meter and electrodes
- 1-mL pipet
- 5-mL pipet
- scanning spectrophotometer
- 12, 25-mL volumetric flasks
- bromothymol blue solution (0.1% in 20% ethanol)
- hydrochloric acid (concentrated)
- KH_2PO_4 solution (0.10 M)
- Na_2HPO_4 solution (0.10 M)
- sodium hydroxide solution (4 M)

PROCEDURE

1. Use a pipet to deliver 1.00 ml of the bromothymol blue solution to each of two, labeled, 25-ml volumetric flasks. Add 5 ml of distilled or deionized water and 4 drops of concentrated hydrochloric acid to one of the flasks. Dilute the solution to the mark with water. The

resulting solution should have a pH of about 1. To the second flask add 12 drops of 4 M sodium hydroxide solution and fill the flask to the mark with water. The solution should have a pH of about 13.

2. Label 10, 25-ml volumetric flasks with numbers 1 through 10. Use a pipet to deliver 1.00 ml of the bromothymol blue solution to each of the flasks. Add the volumes of the 0.10 M Na_2HPO_4 solution and the 0.10 M KH_2PO_4 solution to each flask that are indicated in the table. Dilute each solution to the mark with water. Buffer solutions which have different pH's are prepared in this step.

Flask Number	mL KH_2PO_4	mL Na_2HPO_4
1	5.0	0.0
2	10.0	0.5
3	5.0	1.0
4	10.0	5.0
5	5.0	5.0
6	5.0	10.0
7	1.0	5.0
8	1.0	10.0
9	0.5	10.0
10	0.0	5.0

3. Use the pH meter to measure the pH of the pH 1, hydrochloric acid solution, the pH 13, sodium hydroxide solution, and the 10 solutions which were prepared in this step. Record the pH of each solution.

4. Using the scanning spectrophotometer, obtain and record spectra between 300 and 800 nm of the pH 1 and the pH 13 bromothymol blue solutions. Your instructor will show you how to use the instrument. From the recorded spectra choose one wavelength (λ1) at which the pH 1 solution absorbs strongly but the pH 13 solution absorbs weakly, and a second wavelength (λ2) at which the pH 13 solution absorbs strongly but the pH 1 solution absorbs weakly. Remember, high absorbance corresponds to low percent transmittance.

5. Measure and tabulate the absorbance of each of the 12 solutions at the two chosen wavelengths. It is not necessary to print out a spectrum for the buffered solutions.

Calculations

1. Prepare a plot of absorbance (y axis) as a function of pH (x axis) for the 12 solutions at each of the two wavelengths. Connect the points with a smooth line and determine a value of the pK_a of the indicator from the inflection point in each plot.

2. From each of the two plots, determine a value of the minimum absorbance at each wavelength. Except in cases where an error was made in one of the measurements, the minimum absorbance corresponds to that of the pH 1 solution for 2 and to that of the pH 13 solution for 1.

3. Subtract the minimum absorbance at each wavelength from the absorbance of each of the 12 solutions at the wavelength. The resulting absorbances are corrected for background absorbance.

4. Determine the corrected absorbance in acid solution at λ_1 (A $_{\lambda1,acid}$) and the corrected absorbance in basic solution at λ_2 (A $_{\lambda2,basic}$). These absorbances are the corrected absorbances which correspond to the level portion at the top of each plot (see the Figure).

5. Using equation (13) and the corrected absorbances calculate the ratio [In⁻]/[HIn] at each of the 12 pH's.

6. Plot pH (y axis) as a function of log ([In⁻]/[HIn]). Draw the best possible straight line through the data points and determine the pK_a of bromothymol blue from the intersection of the line with the pH axis.