Spectrophotometric Determination of an Equilibrium Constant[1]

Authors: B. K. Kramer, B. D. Lamp, D. L. McCurdy* and J. M. McCormick
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Introduction

Acid-base indicators are themselves weak acids or bases whose acid and base forms have different colors in solution. As the result of the reaction with excess titrant, we convert one form to the other causing a color change that indicates the endpoint of a titration. (Remember that you used phenolphthalein in Week 2 of the Oil of Wintergreen experiment. In acidic solution it was colorless, but when $[H^+]$ was sufficiently low, it was pink.) If we represent the indicator's acid form as HIn and its basic form as In^-, then the following equilibrium describes the chemical reaction that occurs as the $[H^+]$ is changed. If HIn and In^- have different colors, then the solution's color will change as a function of $[H^+]$ depending on which of the compounds is present in the greater amount.

$$HIn\,(aq) \rightleftharpoons H^+\,(aq) + In^-\,(aq)$$

The acid dissociation equilibrium constant (K_a) for the indicator that describes this reaction is given by Eqn. 1, in terms of the concentrations of the hydrogen ion, In^- and HIn. Because we are working in aqueous solution, it is convenient to rearrange Eqn. 1 to Eqn. 2 by taking the negative base-ten logarithm of both sides. By convention, we use the prefix "p" to denote the negative base-ten logarithm, and so $-\log K_a$ becomes pK_a and the $-\log[H^+]$ becomes pH. This leads to a very convenient way of writing the very small $[H^+]$ that occur in aqueous solution as numbers that generally fall between 0 and 14. We can rewrite Eqn. 2 as Eqn. 3. Note that Eqn. 3 predicts that the indicator's pK_a corresponds to the pH of an indicator solution when the logarithmic term equals zero (i. e., when $[In^-]$ equals [HIn]).

$$K_a = \frac{[H^+][In^-]}{[HIn]} \tag{1}$$

$$-log(K_a) = -log[H^+] - log\left(\frac{[In^-]}{[HIn]}\right) \tag{2}$$

$$pK_a = pH - log\left(\frac{[In^-]}{[HIn]}\right) \tag{3}$$

A convenient way to determine the equilibrium constant of a reaction involving colored species and H^+ is to use absorbance spectroscopy. If we monitor a wavelength at which either one of the two species strongly absorbs we will see the absorbance as a function of pH change as that species' concentration in solution changes. From the equilibrium between HIn and In^-, given above, and considering Le Chatelier's principle, we can see that when the $[H^+]$ is large (low pH), the equilibrium will shift completely to the left and the indicator will be completely in the HIn form. [The somewhat counterintuitive relationship between pH and $[H^+]$ is a result of defining the pH as the negative logarithm of $[H^+]$. As the $[H^+]$ decreases, the exponent portion of the number (when written in scientific notation) becomes a bigger negative number. Thus, the pH becomes a larger positive number.]

Consider the experiment whose results are shown in Fig. 1. In this experiment, the absorbance of a solution of the indicator bromthymol blue is measured as the solution's pH is varied. As the solution pH changes from acidic to basic, we observe an evolution from spectra where the peak centered around 435 nm (denoted by λ_1) is largest, to spectra where the peak at about 620 nm (denoted by λ_2) dominates. A slight complication is that the minimum for the most basic solution in the region of λ_1 is at 450 nm. To minimize the effect of correction factors and to maximize sensitivity of our method, these wavelengths should be the same. Since the wavelengths are a little different, but the peaks (and minima) are somewhat broad, we have a small range of wavelengths which we could choose for the first wavelength. It is most convenient if the whole lab section chooses the same λ_1, either 435 or 440 or 445 or 450 nm. [Further examination of the spectra reveals one wavelength, noted by a star, where the absorbance is essentially independent of pH. This is called an isosbestic point and results from the both forms of the indicator (HIn and In^-) having the same molar absorptivity at this wavelength. It is not uncommon to see one or more isosbestic points in a set of spectra.]

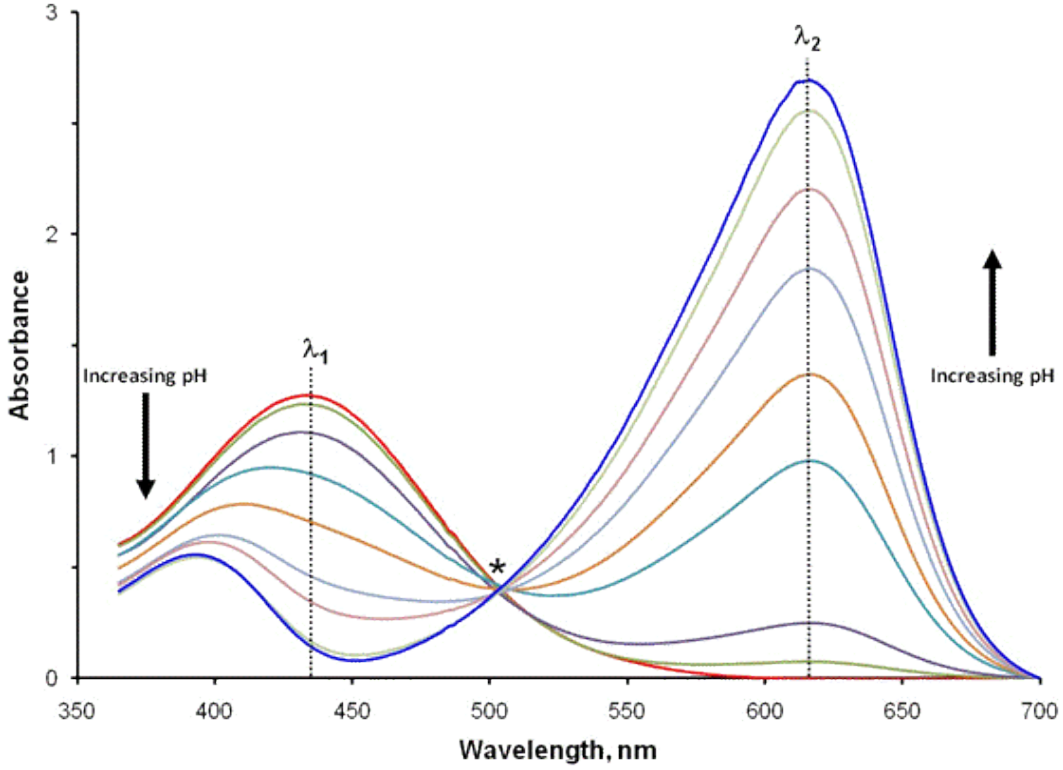

Figure 1. Spectra of the indicator bromothymol blue as a function of pH.

A closer examination of the trends in the spectra is shown in Fig. 2. When the solution is very acidic, as on the left side of Fig. 2, all of the indicator is in the HIn form, resulting in a large absorbance at λ_1 (labeled $A_{max,\lambda 1}$) but a small absorbance at λ_2 (since $[In^-]$ is small). At high pH, all of the indicator is in the In^- form giving a strong absorbance at λ_2 (labeled $A_{max,\lambda 2}$) and minimal absorbance at λ_1. As the pH changes from acidic to basic, the position of the equilibrium changes such that HIn is converted to In^- in accordance with Eqn. 3. This

conversion results in a decrease in [HIn] and a corresponding increase in [In^-]. Since the absorbance at each wavelength is directly proportional to concentration, we observe a decrease in the absorbance at λ_1 (because [HIn] decreases), and an increase in the absorbance at λ_2 (because [In^-] increases). It is important to realize that even though we may only be collecting absorbance values at two wavelengths in our experiment, the entire spectrum is undergoing a transformation as we cause the relative amount of HIn to decrease and In- to increase by increasing the pH of the system. In principle, we could choose any wavelength where HIn absorbs for λ_1, and any wavelength where In^- absorbs for λ_2 in our analysis. In practice, however, it is beneficial to choose the wavelength of maximum absorbance for each species. Doing so provides the greatest sensitivity (ability to distinguish small changes in absorbance) for our measurement.

From Eqn. 3 is should be obvious that the pH where [HIn] = [In^-] corresponds to the indicator's pK_a. This occurs when exactly half of the indicator is in the HIn form and half is present as In^-. In terms of the experiment, this corresponds to the pH where the absorbance for each form is one half of its maximum, as shown by the dotted line in Fig. 2. Consequently, the pK_a of an indicator corresponds to the pH of the solution at the inflection point in a plot of absorbance as a function of pH.

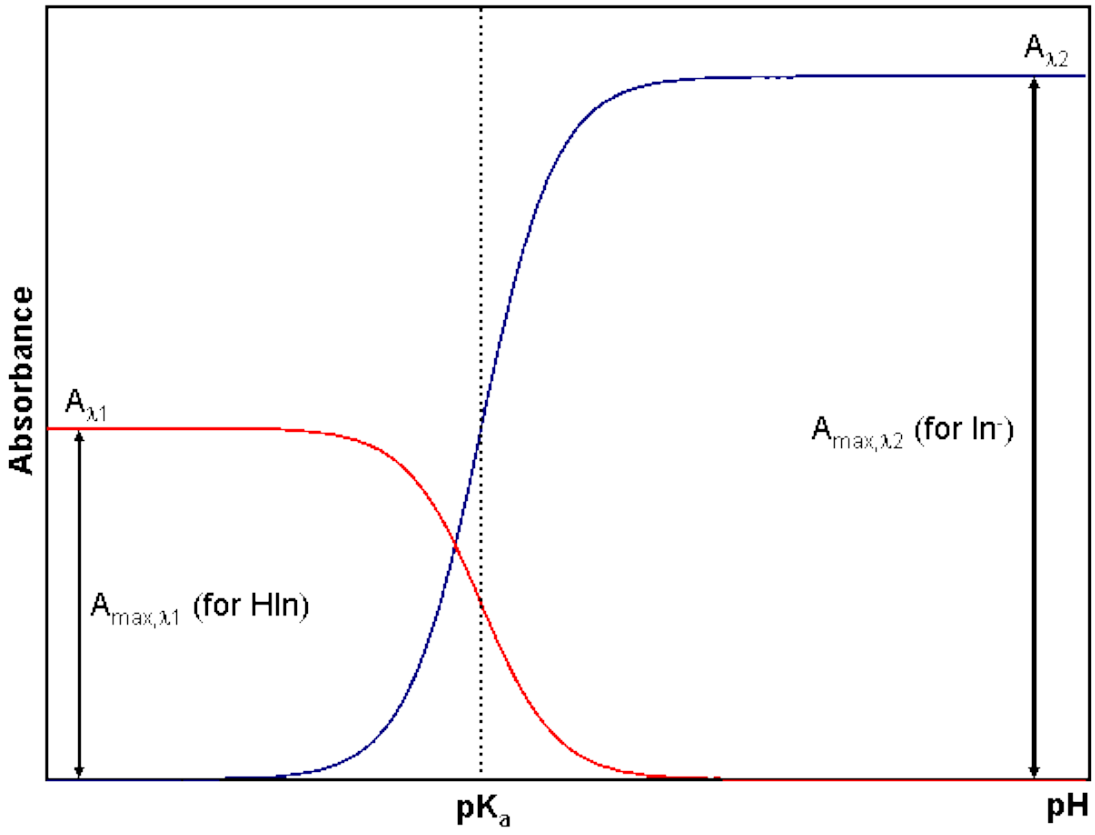

Figure 2. Dependence of absorbance HIn and In^- on pH. The red line shows how the absorbance changes at a wavelength (λ_1) where the acidic form of the indicator, HIn, absorbs strongly. The blue line indicates the behavior at a wavelength (λ_2) where In^-, the basic form of the indicator, absorbs strongly. The pH at which the inflection point in both lines occurs is the indicator's pK_a.

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

In practice it is difficult to precisely and accurately determine the inflection point in curves of this type. To get a more precise measure of the pK_a, Eqn. 3 is rearranged to give Eqn. 4. This equation gives a straight line when the solution's pH is graphed as a function of log([In^-]/[HIn]). The slope of this line should be +1 and the *y*-intercept, where log([In^-]/[HIn]) is zero (i. e. [In^-] = [HIn]), is the pK_a, as shown in Fig. 3.

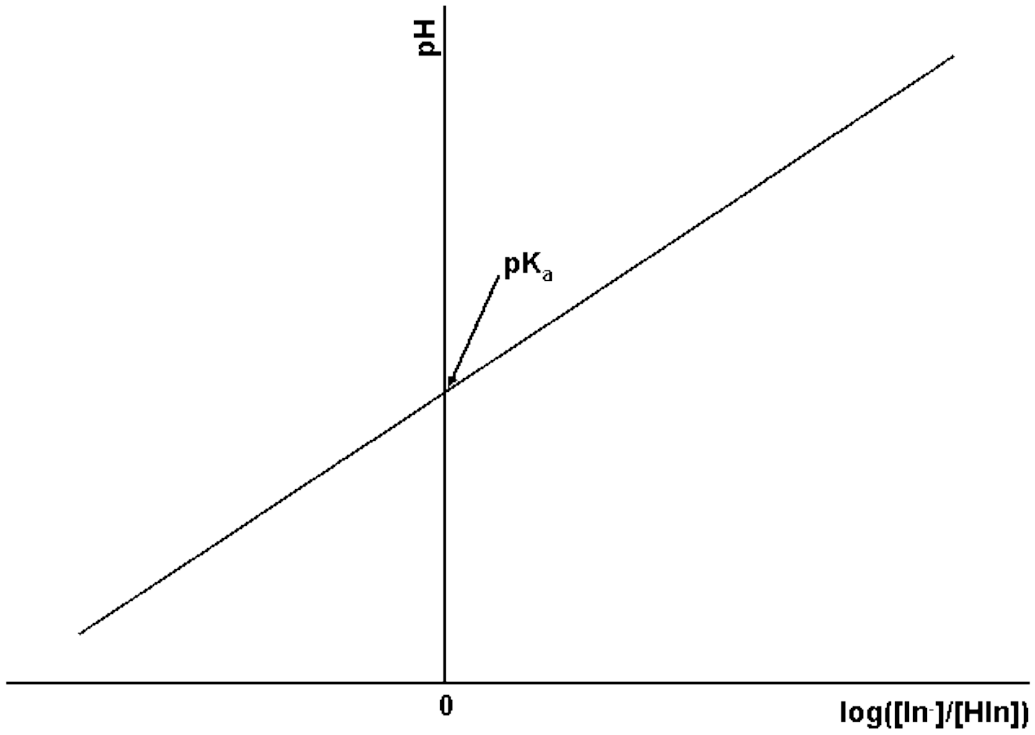

Figure 3. Relationship between pH and log([In^-]/[HIn]) for an indicator. The pK_a of the indicator corresponds to the intersection of the line with the pH axis.

To use Eqn. 4 to determine the pK_a of the indicator, it is necessary to know the pH of solutions that have different ratios of the two indicator species HIn and In^-. Since the pH of the solution will determine the amount of the total indicator that will be in each form, it can be difficult to control exactly how much of the indicator exists as In^- and HIn. It is possible, however, to use absorbance to obtain the ratio [In^-]/[HIn]. We will simply monitor the absorbance at two different wavelengths. The first wavelength (λ_1) is chosen where the acidic (HIn) but not the basic (In^-) form of the indicator strongly absorbs radiation. The second wavelength (λ_2) is chosen where the basic but not the acidic form strongly absorbs radiation.

$$A_{\lambda 1} = \varepsilon_{(HIn,\lambda 1)} \cdot b \cdot [HIn] \qquad (5)$$

If Beer's law is obeyed, the absorbance at λ_1 is given by Eqn. 5 where $A_{\lambda 1}$ is the absorbance at ε_1, $\lambda_{HIn,\lambda 1)}$ is the molar absorptivity of HIn at λ_1 and b is the cell path length. Since the amount of the indicator that is in the form of HIn depends on the pH, [HIn] can be difficult, or impossible, to determine. What is known, however, is the total concentration (C_T) of the indicator in both forms since a known amount of the indicator was added to the solution at the beginning. At any pH the indicator's C_T is given by Eqn. 6. In solutions where the pH is sufficiently low, all of the indicator is in the acidic form, and consequently C_T = [HIn]. Substituting C_T = [HIn] into Eqn. 6 gives Eqn. 7.

$$C_{tot} = [HIn] + [In^-] \tag{6}$$

$$A_{\lambda 1}(lowest\ pH) = \varepsilon_{(HIn,\lambda 1)} \cdot b \cdot C_T \tag{7}$$

Likewise, at λ_2 we can write an equation analogous to Eqn. 6 (Eqn. 8) where we have simply replaced the molar absorptivity of HIn with that of In^- and the concentration of [HIn] with [In^-]. In basic solution, C_T = [In^-], which leads to Eqn. 9.

$$A_{\lambda 2} = \varepsilon_{(In^-,\lambda 2)} \cdot b \cdot [In^-] \tag{8}$$

$$A_{\lambda 2}(highest\ pH) = \varepsilon_{(In^-,\lambda 2)} \cdot b \cdot C_T \tag{9}$$

The ratio [In^-]/[HIn] at any pH can be obtained by combining Eqn. 5 and Eqn. 8 to give Eqn. 10. Substituting equations Eqn. 7 and Eqn. 9 into equation Eqn. 10 gives Eqn. 11, which simplifies to Eqn. 12.

So the first step of our experiment will be to determine the value of $A_{\lambda 1}$(lowest pH) and $A_{\lambda 2}$(highest pH). $A_{\lambda 1}$(lowest pH) is the maximum absorbance at λ_1 ($A_{max,\lambda 1}$) in the solution with the lowest pH which should be only due to HIn. $A_{\lambda 2}$(highest pH) is the maximum absorbance at λ_2 ($A_{max,}\lambda_2$) in the solution with the highest pH and it should be due to only In^-.

$$\frac{[In^-]}{[HIn]} = \frac{\left(\frac{A_{\lambda 2}}{\epsilon_{In^-\lambda 2} \cdot b}\right)}{\left(\frac{A_{\lambda 1}}{\epsilon_{HIn\lambda 1} \cdot b}\right)} \tag{10}$$

$$\frac{[In^-]}{[HIn]} = \frac{A_{\lambda 2}\left(\frac{C_T}{A_{\lambda 2}(highest\ pH)}\right)}{A_{\lambda 1}\left(\frac{C_T}{A_{\lambda 1}(lowest\ pH)}\right)} \tag{11}$$

$$\frac{[In^-]}{[HIn]} = \frac{A_{\lambda 2}/A_{\lambda 2,highest\ pH}}{A_{\lambda 1}/A_{\lambda 1,lowest\ pH}} \tag{12}$$

Until this point we have assumed that at λ_1 the measured absorbance is due only to any HIn present and at λ_2 all absorbance is due to In^-. In fact, the basic form may absorb somewhat at λ_1 and the acidic form may absorb at λ_2. Because of this, the values of absorbance used in these equations must be corrected to take into account the amount of the absorbance that is due to the other species. To make this correction, we simply subtract the minimum absorbance measured at λ_2 from each of the other measurements made at that wavelength. For example, $A_{\lambda 2}$ = $A_{\lambda 2}$(measured) - $A_{\lambda 2}$(mimimum), where the value of $A_{\lambda 2}$(mimimum) comes from the measurement made on the most acidic solution at λ_2, the wavelength at which it should have the least absorbance. The same correction is to be made for λ_1 by measuring the absorbance of the most basic solution at λ_1.

Experimental

In this experiment, you will determine the pK_a of bromothymol blue (3',3"- dibromo-thymolsulfonephthalein) by the two methods which have been discussed. At a pH which is less than 6, the indicator is yellow and at a pH which is greater than 7.6, the indicator is blue. At an intermediate pH, the blue and yellow combine to yield a green solution. You will use the Spectronic 20 Genesys spectrometer to measure the absorbance of a bromothymol blue solution at the two specified wavelengths as a function of pH. You will also be using a pH electrode interfaced to Logger Pro A/D converter (LabQuest) to measure the pH of each solution that you make. There will be two or three spectrometers in the lab, so you will be sharing with other tables.

Before coming to the laboratory prepare a table in Excel (landscape orientation) with the ten column headings shown below:

Flask Number, Absorbance at 435 nm, Absorbance at 620 nm, Solution Color, pH, Corrected Absorbance at 435 nm, Corrected Absorbance at 620 nm, $[In^-]/[HIn]$, $\log\{[In^-]/[HIn]\}$, pH

Enter numbers 1 through 9 in the nine rows below "Flask Number."

As soon as you get into the laboratory, check out one tote per table from the stockroom with your Truman ID.

Label nine 25-mL volumetric flasks as "Flask 1" through "Flask 9". Use a volumetric pipet to deliver 1.00 ml of the bromothymol blue solution to each of the flasks. To "Flask 1" add 5 mL of distilled or deionized water and 4 drops of concentrated hydrochloric acid. Dilute the solution to the mark with water. The resulting solution should have a pH of approximately 1. To "Flask 9" add 12 drops of 4 M sodium hydroxide solution (**CAUTION!** the sodium hydroxide solution is very caustic) and fill the flask to the mark with water. The solution should have a pH of about 13. 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 Table 1 using graduated cylinders. Dilute each solution to the mark with water.

Flask Number	Volume of KH_2PO_4 Solution (mL)	Volume of Na_2HPO_4 Solution (mL)
2	5.0	0.0
3	5.0	1.0
4	10.0	5.0
5	5.0	10.0
6	1.0	5.0
7	1.0	10.0
8	0.0	5.0

Table 1. Volumes of 0.10 M KH_2PO_4 solution and 0.10 M Na_2HPO_4 solution to be added to each volumetric flask.

Prepare the Spectronic 20 Genesys spectrometer to obtain absorbance readings at 435 nm or at 620 nm according to the instrument's operating instructions. Since you have to run a blank each time after you change the wavelength, take all nine of your solutions to the Spec 20. Choose a wavelength, run a blank, and then measure the absorbance of all nine solutions, rinsing several times with the new solution each time before measuring the absorbance. Change to the other wavelength, run a blank, and measure the absorbance of the nine solutions at the second wavelength. Work collegially and effectively with other groups sharing a particular spectrometer. Record your 18 absorbance values in your Excel table. Use the same cuvette throughout this experiment! Remove any bubbles from the cuvette by gently tapping with your finger. **Under absolutely no circumstances are you to tap a cuvette on a table top.**

Set up and calibrate the pH electrode with the pH electrode interfaced to an A/D converter. Directions start on page 98 if you use Logger Pro on your computer to control a LabQuest instead of a LabPro; directions start on page 80 if you only use the LabQuest. Measure the pH of the solutions in all nine flasks, and record the pH of each solution in your Excel table. Be sure to copiously rinse the pH electrode with distilled water between each measurement and *pat* (do not rub) the electrode dry. When you have finished your measurements, place the electrode in the standard pH 7 buffer solution that you used to calibrate the electrode.

Prepare both graphs discussed in the next section and show them to your professor before discarding any of your solutions.

Results and Analysis

In Excel subtract the minimum absorbance at each wavelength (the minimum absorbance should correspond to the absorbance of the flask 1 solution at λ_2 and to that of the flask 9 solution at λ_1) from the absorbance of each of the nine solutions at that wavelength (use an absolute cell reference). The resulting absorbance values are now corrected for background absorbance. Prepare a graph of absorbance as a function of pH for the nine solutions at each of the two

wavelengths. This should look like Figure 2. Figure 2 is an idealized graph and does not show any actual data points. In your version of Figure 2 based on your data, show data points without any connecting lines. Show major grid lines every one pH unit and minor grid lines every 0.5 pH units. Show major grid lines for the corrected absorbance every 0.1 absorbance unit. In the legend on your chart, rename Series1 and Series2 as perhaps 435 nm and 620 nm. Mentally 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. It is up to you to determine the best way to find the inflection points.

Using Eqn. 12 and the corrected absorbances calculate the ratio $[In^-]/[HIn]$ at each of the nine pHs (use a formula in Excel). Graph pH as a function of log ($[In^-]/[HIn]$), as in Fig. 3. From the best-fit line through the data determine the *y*-intercept and thus the pK_a of bromothymol blue. Share your value with your laboratory section. If your professor desires, perform a Q-test on the class data, and discard an errant datum, if warranted. Calculate the estimated standard deviation on the class average pK_a and the confidence limits on the average pK_a at the 95% confidence level.

Conclusions

Use the outline for a measurement laboratory as a guide as your write your conclusions.

References

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