Identification of an Unknown Indicator by Spectrophotometry Using Two pK_a Calculation Methods

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The Cooper Union
for the Advancement of Science and Art

CH-111 Section C
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November 19th, 2018

CONTENTS

ABSTRACT

Each substance transmits and absorbs a fixed amount of light at a certain wavelength; when plotting absorbance as a function of wavelength, the substance's absorption spectrum is obtained. Spectrophotometry is a specific form of electromagnetic spectroscopy used to determine the identity of substances by measuring their absorption spectra using a spectrophotometer. Spectrophotometry was used to generate absorption spectra for three buffered solutions of the unknown indicator #1815 at different pH levels, as well as to generate Beer's law plots of absorbance versus concentration at peak wavelength values of the indicator. The absorption spectra and the Beer's law plot were used to calculate the pK_a of the indicator by two different calculation methods (one using absorption directly, and the other focusing on concentrations), which was then used to determine the identity of the indicator. Method 2 was deemed more reliable, and it produced a calculated pK_a value of 4.13. The properties of the indicator closely matched the literature values of the bromophenol blue indicator, which has a literature pK_a value of 4.10. Because the standard deviation of the experiment was so low and the calculated pK_a value was so close to the literature value, this spectrophotometry procedure (and pK_a calculation method 2) could be used to identify different substances by their absorbance.

EXPERIMENTAL METHODS

Spectrophotometry was used to determine the identity of an unknown pH indicator. Absorption spectra of three buffered solutions at different pH levels (one below the effective pH range, one in the effective pH range, and one over the effective pH range) were measured using a spectrophotometer and plotted. Optimal wavelengths from the absorption spectra were used to measure the absorbance of different concentrations of the low and high pH buffered solutions to form a Beer's law plot. The absorption spectra and Beer's law plot were then used to estimate the pK_a of the unknown indicator by two calculation methods. The procedure followed was based on the procedure stated in "Spectrophotometric Determination of the Identity of an Unknown Indicator," from *The Official Cooper Union General Chemistry Laboratory Guide* (1). The discussion behind the methods used are explained in more detail in the discussion section.

The indicator was put in acetic acid, $HC_2H_3O_2$ (aq), solution, and its pH was raised by adding sodium hydroxide, NaOH (aq), until the color stopped changing, in order to determine the effective pH range of the indicator. Buffered solutions at a pH of roughly 2 pH units below and above the effective pH range bounds, and a buffered solution in the middle of the pH range, were created to a known concentration. The pH measurements were taken using the Fisher Scientific AB150 pH meter. The pH meter was calibrated before use using an automatic 3-point calibration built into the AB150 pH meter, automatically recognizing the pH 4, pH 7, and pH 10 standard buffer solutions; it was not re-calibrated before measuring the pH of the three prepared buffered solutions because the digital 3-point automatic calibration is more robust than the 2-point, manual calibration outlined in the laboratory manual. Absorption spectra of each of the three buffered solutions were recorded at mostly consistent intervals from 350nm to 650nm wavelengths, and with measurements clustered more closely near the peaks. Optimal wavelengths for the two species (low pH and high pH) were identified. The low pH and high pH solutions were diluted to several concentrations, and a Beer's law plot was obtained by measuring the absorbance of both solutions at their respective optimal wavelengths. The spectrophotometer used was the Thermo Spectronic Genesys 20.

A potential gross error is that, due to inexperience with the pH meter, the tip of the pH meter was not always submerged in the provided equipment buffer solution or in a solution to be measured. Another potential source of error is that the cuvettes were not exactly matched. When filling the four cuvettes with water and measuring the absorbances (to check that the cuvettes were matched), one cuvette had an absorbance value 0.002 higher than the others. Other possible sources of gross error are the formation of bubbles in the cuvettes, the analysis of cuvettes that were not rotated equally in the spectrophotometer, the analysis of cuvettes that are not completely dry, and touching cuvettes with gloves in the region of the cuvette that was analyzed spectrophotometrically. Due to lack of experience with the spectrometer, the cuvettes were

touched with (gloved) hands below the analysis point several times at the beginning of the procedure, especially when washing the cuvettes. A best attempt was made at clearing any glove residue off by washing and drying with Kimwipes. Solutions with noticeable bubbles were discarded, but it is possible that small bubbles too small to notice, but large enough to affect the spectrophotometer reading, were left behind. These potential sources of error are discussed in greater detail in the discussion.

RESULTS

The effective pH range was determined by placing the indicator in a solution with an initial pH of 2.4, and increasing the pH by adding sodium hydroxide until the full color change is observed. The complete recorded data of color versus pH is in Appendix III, Table 7. The effective pH range (and the associated colors of the bounds of the effective pH range) is displayed in Table 1.

Table 1. Effective pH Range and Colors

	pH	Color
Low end of effective range	3.2	yellow
High end of effective range	4.2	violet

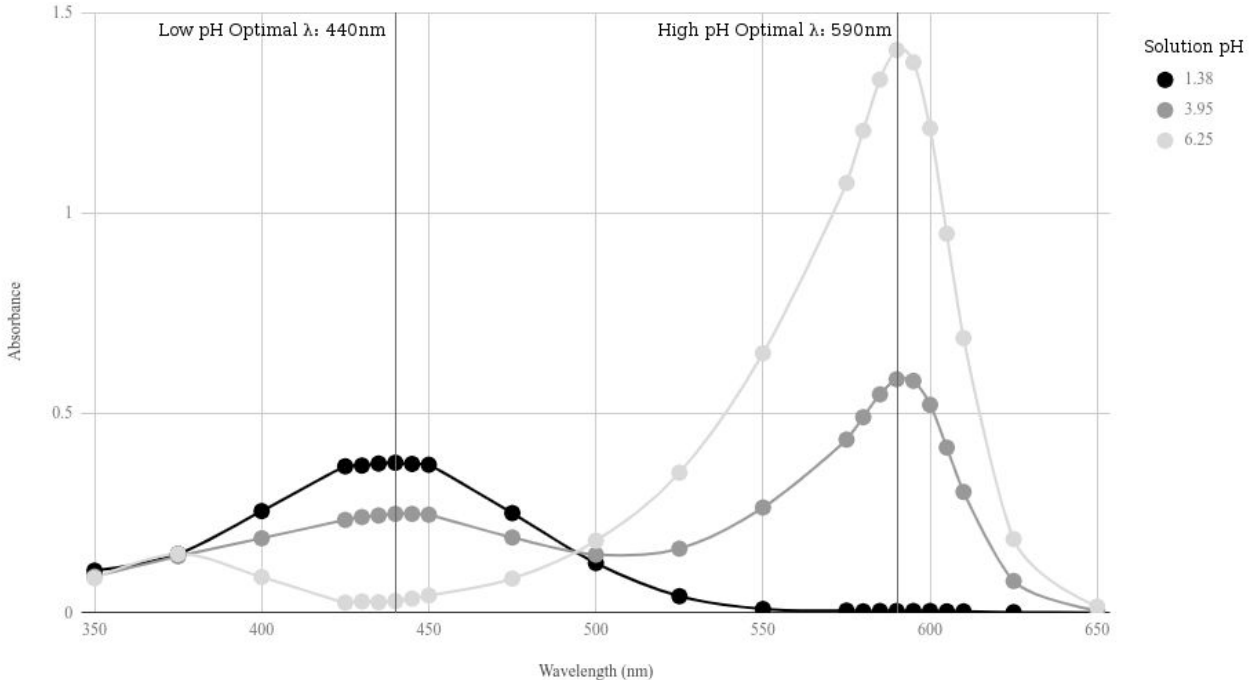
The pH values of the three prepared buffered indicator solutions are displayed in Table 2. The low pH solution was created to be roughly 2 pH units below the low bound of the effective pH range, the high pH solution was created to be roughly 2 pH units above the high bound of the effective pH range, and the pH of the intermediate pH solution was placed in the effective pH range.

Table 2. pH and Optimum Wavelength of Buffered Indicator Solutions

	pH	Color	Optimum Wavelength (nm)
Low pH	1.38	yellow	440
Intermediate pH	3.95	dark violet/red	—
High pH	6.25	violet	590

The absorption spectra of each solution was measured and graphed in Figure 1. The peak values for the low and high pH solutions are indicated on the graphs and recorded in Table 2. These absorption spectra data can be found in textual form in Appendix III, Table 8.

Figure 1: Absorption Spectra of Three pH Indicator Buffered Solutions



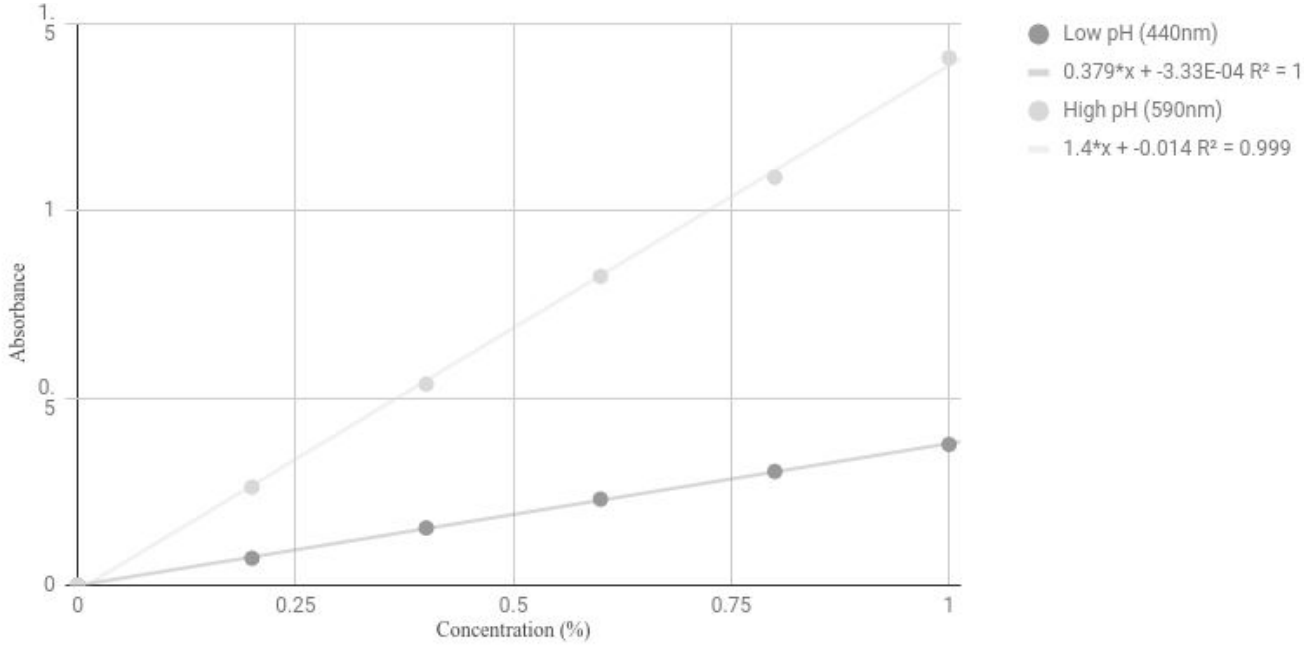
The measured Beer's law data (absorbance versus concentration) of the low and high pH solutions at their optimal wavelengths are displayed in Table 3. The 100% concentration value is the absorbance value from the absorption spectra of the two solutions at their optimal wavelengths. The 0% concentration is an added theoretical point used to strengthen the correlation, because a zero concentration should have zero absorbance.

Table 3. Absorbance vs. Concentration at Optimum Wavelengths for Low and High pH

	Absorbance	
Concentration (%)	Low pH (440nm)	High pH (590nm)
0	0	0
20	0.072	0.262
40	0.153	0.537
60	0.230	0.825
80	0.304	1.090
100	0.376	1.409

The data from Table 3 is displayed graphically in Figure 2. The coefficient of determination (r^2 value) for both linear regressions is very high (1.00 and 0.999 for the low pH and high pH solutions, respectively). The coefficient of determination represents the percent of the variation in the absorbance that is explained by the variation in concentration; the 1.00 and 0.999 mean that roughly 100% and 99% of the variation in absorbance is caused by a variation in concentration, or that there is a very strong linear correlation. By Beer's law, the absorbance should be proportional to concentration, so this is expected.

Figure 2. Absorbance vs. Concentration at Optimum Wavelengths for Low and High pH



The proportionality constant for the relationship between absorbance and concentration for the high and low pH solutions is the slope of the corresponding trendlines on Figure 2. Because both absorptivity (a) and length of light path (b) are constant for each substance, the proportionality constant is $a_{substance}{}^{\lambda}b$, where substance is HIn or In^- at a specific wavelength. These slopes correspond to the values $a_{HIn}{}^{440nm}b$ (slope of low pH Beer's law graph at low pH optimum wavelength) and $a_{In^-}{}^{590nm}b$ (slope of high pH Beer's law graph at high pH optimum wavelength). These values are stated in Table 4 and used in method 2.

The other two values listed in Table 4 are approximations for the ab products of the non-dominant indicator species at the optimal values. Because the concentrations are so small, a reasonable approximation for these ab values are the absorbance values of the non-optimal solution at the specified wavelength. For example, the approximation for $a_{HIn}{}^{590nm}b$ is 0.030, the absorbance of HIn at 590nm.

Table 4. Beer's Law Absorptivity-Length of Light Path (ab) Products

	Wavelength	
	440nm	590nm
$a_{HIn}\ b$	0.379	0.005
$a_{In^-}\ b$	0.030	1.402

A summary of the pK_a value determined from the two methods are stated in Table 5. It is apparent that the mean pK_a calculated from method 1 is the same as the mean value calculated by method 2 (to the appropriate number of significant figures). These values are obtained from the calculations in A.1.2. And A.1.3., and the mean value for method 1 is obtained from Appendix 2.

Table 5. Summary of pK_a Values

	pK_a
Experimentally determined (method 1 mean)	4.13
Experimentally determined (method 2)	4.13
Literature value	4.10

Statistical measures for the determined pK_a values are displayed in Table 6. While method 1 calculated a pK_a value at every wavelength, method 2 only resulted in 1 pK_a calculation; therefore, method 1 involves a mean, standard error, and standard deviation for the pK_a, while method 2 does not have either. The statistics are obtained in Appendix 2.

Table 6: Methods 1 and 2 Statistical Measures

	Mean	Standard Deviation	% Error
Method 1	4.13 ± 0.0107	0.0477	0.8%
Method 2	—	—	0.6%

For method 1, the standard error and standard deviation of the data were very small (0.0107 and 0.0477, respectively). Both methods calculated very similar pK_a values, which had very small percent error values (< 1%). This suggests that the error in the experiment was very small, and that the results (from either method) were very reliable.

DISCUSSION

Much of the insight on the discussion of the experimental method was based off of content from *Fundamentals of Analytical Chemistry* (2).

pH indicators are compounds that can form a weak acid, notated generically as HIn, and a weak (conjugate) base, notated In^- (1). These two forms of the indicator have different colors; when the ratio of the two shifts, the color of the indicator changes. The net ionic reaction for the hydrolysis of the indicator acid is shown below.

Reaction 1: $H_2O_{(l)} + HIn_{(aq)} \rightleftharpoons H_3O^+_{(aq)} + In^-_{(aq)}$

The acid dissociation constant (K_a) of the indicator acid is the dimensionless product of the concentrations of the products divided by the concentration of the indicator acid. The pK_a of the acid can be calculated using the pH of the solution and the ratio of the concentration of In^- to the concentration of HIn, using the Henderson-Hasselbalch equation (see A.1.1. Equation 1); this is useful because of the pH of the solutions are known and the ratio of the concentration of In^- to the concentration of HIn can be approximated and calculated by methods 1 and 2 (see A.1.2. and A.1.3. for full calculations).

When the pH of the solution is low, then the $H_3O^+_{(aq)}$ concentration is high. By Le Chatelier's principle, the equilibrium shifts left as a result because the reverse reaction is favored, creating a higher concentration of the indicator acid and a lower concentration of its basic form. The opposite is also true: when the pH is high, then the $H_3O^+_{(aq)}$ concentration is low, and the equilibrium shifts right by Le Chatelier's principle by favoring the forward reaction, causing the concentration of the basic form to increase and the acidic form to decrease.

Thus, the color of the indicator is the color of HIn at a low pH; the color of the indicator is the color of In^- at a high pH. When the pH is low or high enough, one form of the indicator dominates, and the color stops changing when the pH becomes more extreme; the "effective range" of the indicator is the pH range when neither form of the indicator dominates (and thus the color changes in response to changes of pH).

The indicator solutions are pH-sensitive, so they are made into solution with a buffer, potassium dihydrogen phosphate, KH_2PO_4 (aq), before being analyzed. A buffered solution comprises of the salt of a weak acid and its weak conjugate base. This works by Le Chatelier's principle as well: adding acid to the buffer solution will favor the reaction that forms the conjugate base, and adding base to the solution will favor the reaction that forms the conjugate acid, keeping the

acidity relatively constant. The buffer also resists changes to acidity when moderately diluted: dilution will decrease the concentration of hydronium ions, which will cause the system to favor the reaction that produces more hydronium ions by Le Chatelier's principle. As long as the hydronium ions are not excessively depleted, the pH should remain relatively constant. The ability of the buffer to resist changes in pH when diluting is important because the pH was modified to a desired level (two units below the low bound of the effective pH range, the center of the effective pH range, and two units above the high bound of the effective pH range) by adding acid or base before dilution to 100mL of solution. After dilution the pH remained close to the desired pH.

On the plotted absorption spectra, all three absorbances met at the isosbestic point, the point at which absorbance is independent of pH. This point occurs because both indicator species absorb that wavelength of light equally; because the total concentration of indicator species is constant, the total absorbance is equal no matter the pH because the relative amounts of the two indicator species does not matter. This point is significant because the pK_a cannot be calculated from this point using the approximation in method 1 (see A.1.2. Equation 2): would result in the division of zero ($A_{int.} - A_{low}$) by zero ($A_{high} - A_{int.}$), an indeterminate number. The isosbestic point appears around the 490nm wavelength for the indicator (Figure 1). This does not cause a problem with these results because there were no absorbances measured very close to that wavelength, and therefore no division-by-zero indeterminate values.

Two methods were used to calculate the pK_a of the indicator. The first method used the approximation stated and derived in A.1.1. Equation 2 that uses the absorbances of all three solutions at a given wavelength to calculate the ratio of concentrations of $[In^-]$ to [HIn] in the intermediate pH buffered solution at any wavelength (except for the isosbestic point).

Using this method and this equation, a few possible extraneous values for the concentration ratio may be obtained. A zero value means that the numerator of the expression is zero (and the denominator is not); this means that the absorbances of the low and intermediate pH solution are equal, but different from the absorbance of the high pH solution. An indeterminate value is possible at the isosbestic point, as mentioned above. A negative value occurs if the absorbance for the intermediate value is not truly intermediate between the low pH and high pH solution absorbances. The isosbestic point should not be considered because

The second method relies on the fact that the absorbance of a solution with two series is equal to the sum of the absorbances of the two species individually. This is expressed in A.1.3. Equation 4 as the sum of two Beer's law expressions— one for each indicator species. A linear system of two equations can be written to solve for the concentrations. The first equation is the sum of the absorbances of the two species at the low pH optimal wavelength; the second equation is the sum

of the absorbances of the two species at the high pH optimal wavelength. Because absorbance is proportional to concentration, the coefficients of the concentrations of the species are the proportionality constant $a_{species}{}^{\lambda}b$. The four ab products are tabulated in the Results Table 4, along with a description of the source of the values. Once the system is solved, the ratio of the concentrations of the In^- to HIn is known, and the pK_a can be solved using A.1.1. Equation 1 in the same way as in method 1. The entire calculation for method 2 is detailed in A.1.3.

The value of b (the length of the light path through the solution in the Beer-Lambert equation) does not have to be found because it should be constant for every sample because the cuvettes were matched. The absorptivity of a solution is also constant for each species (HIn and In^-); because both a and b are constant, they can be combined into one proportionality constant for in the Beer's law plot (i.e., the slope of the absorbance vs. concentration).

There are a few notable sources of error that must be mentioned. A possible systematic error could arise from the cuvettes not being evenly matched. Our third cuvette absorbance was measured to be 0.002 while the others had absorbances of 0.000. This would result in slightly greater absorbance values for our third sample because the optical properties of this cuvette would be slightly different from the others. A random error may happen from the formation of bubbles before the samples are tested. If the sample contains small bubbles, the light going through the cuvette will be refracted and the reading on the spectrometer will be incorrect.

A gross error could arise from the uncertainty that all the cuvettes going into the spectrophotometer may have not been completely dry. If there are droplets of sample on the outside of the cuvette, then as light is incident on the cuvette, it may exhibit different refraction patterns and avoid travelling to the other end of the spectrophotometer. Also, the orientation in which the cuvette is placed into the spectrophotometer is uncertain. Although the cuvettes were always placed in the spectrophotometer so that the symbol on the cuvettes were always facing a certain direction, slight changes in this orientation could still impose a significant random error.

There could also be errors in how the results were measured. Instead of using the buffer to zero the spectrophotometer, deionized water was used. This systematic error could result in an increase in each of the values of absorbance because the spectrophotometer will measure the absorbance of the buffer as well as the indicator sample.

The errors, ranked from most to least significant, are the possibility of droplets on the outside of the cuvettes, improperly rotated cuvettes, a slightly-mismatched cuvette, small bubbles forming, and the inherent uncertainty of the equipment (very small). The fact that the standard deviation of the calculations from method 1 were so small and that the calculated pK_a values from both calculation methods would suggest that the data obtained was reliable and that errors were small.

The unknown indicator was determined to most likely be bromophenol blue. Its literature pK_a value is 4.10 (Appendix 4), which is very close to the 4.13 pK_a calculated from both methods. It has a very similar known pH range (literature values 3.0-4.6 are very close to the observed color range of 3.2-4.2) and color change (literature value of yellow to violet matches experimental observations). These values would make

Both methods incorporate approximations into the calculations. Method 1 used A.1.1 Equation 2, which uses the approximation that the concentration of HIn in the low pH solution is equal to the total concentration of indicator species and that the concentration of In^- is equal to the total concentration in the high pH solution. This approximation is useful because the HIn almost completely dominates in the low pH solution, and the In^- almost completely dominates in the high pH solution. Method 2 used A.1.3. Equation 4. There are two equations and four coefficients: the two ab coefficients of the species at their optimal wavelengths were measured, and the two ab coefficients of species not at their optimal wavelengths were estimated using absorbances (which were very near zero). Method 1 used two approximations per wavelength, but the final value was the mean of many values calculated at different wavelengths, so the total effect from number of approximations should be similar in the two methods.

One important difference between the two is that method 1 may include extraneous points. It was discussed earlier that the isosbestic point will return an indeterminate value for the pK_a. At some wavelengths where the absorbance of the intermediate pH solution is very close to the absorbance of the low or high pH solutions, it is possible that the measured intermediate value does not fall between the high and low pH values, causing a negative ratio of In^- to HIn, and also causing an indeterminate value (because of a negative logarithm; this is similar to the data at 375nm). Even at other wavelengths where the absorbance of the intermediate pH value is strictly in between the absorbance of the lower pH solution and the higher pH solution, if the difference between the high and low pH absorbances is small, anomalously large values may result as the denominator of the approximation approaches zero. However, the second method only uses two equations, each equation representing the absorbance of one of the species is at its peak (and the concentration of the other species is very low), eliminating the chance of indeterminate or anomalous values that could arise in method 1.

Even though the final pK_a results from the two methods were very similar, the second method is likely more reliable. It can be argued that the first point (outlier) and the second point (indeterminate), which were removed from method 1's data set with statistical evidence, were anomalies that could not happen using method 2's calculation for the reasons stated above, which strengthens the claim that method 2 is more reliable.

CONCLUSIONS

Using spectrophotometry and the principles behind Beer's law, the pK_a of the unknown indicator #1815 was calculated to be 4.13 ± 0.0107 by the method of using absorbances, and 4.13 by the method of using the sum of concentrations at two different wavelengths. This compares closely to the 4.10 pK_a literature value of bromophenol blue (Appendix 4), which has a similar known pH range (literature values 3.0-4.6) and color change (yellow to violet).

It was decided that the second method was less reliable than the first method. This was because the calculation was inaccurate or indeterminate when the absorbances of the three solutions were very close to one another (when the wavelength is very low, very high, or at the isosbestic point), and it was indeterminate if the intermediate value was not truly indeterminate to the other values. The procedure for method 1 may be improved by having a determining at which wavelengths the absorbances may too close, and the calculated pK_a values from these wavelengths would be omitted.

If rectangular cuvettes were used instead of cylindrical ones, there may some reduction of error, because they enforce that the cuvettes are oriented exactly the same and remain matched very well. It may also be beneficial to use the buffer solution used in the buffered indicator solutions as the blank instead of water because the absorbance of water and the buffered indicator solution may not be exactly the same.

ACKNOWLEDGEMENTS

The Cooper Union for the Advancement of Science and Art provided the laboratory and safety equipment, as well as the chemicals used in this experiment.

Jonathan Lam was responsible for authoring the Abstract, Experimental Methods, Results, Discussion, References, Appendix I, Appendix II, and Appendix III sections of this laboratory report, and served as editor for the sections written by Paul Cucchiara.

Paul Cucchiara was responsible for authoring the Conclusions, Appendix IV sections of this laboratory report, and served as editor for the sections written by Jonathan Lam.

Andrew Kim was consulted when answering the questions in the Discussion section. This includes some of the calculations in A.1.1.

The format of this laboratory report is based off of the “Laboratory Report” section of The Official Cooper Union General Chemistry Laboratory Guide, 19th edition, by Marcus Lay et al.

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APPENDIX I
DERIVATIONS AND SAMPLE (REPRESENTATIVE) CALCULATIONS

A.1.1. Derivations of Henderson-Hasselbalch and Concentration Ratio Approximation

The Henderson-Hasselbalch equation is used to calculate pKa from the pH of the solution and the ratio of the equilibrium concentration of the conjugate base to the equilibrium concentration of the acid. The derivation follows the equation.

Equation 1 (Henderson-Hasselbalch Equation). $K_a = \frac{[H^+][In^-]}{[HIn]}$

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

This equation is used in A.1.2. and A.1.3. to calculate the pKa from the intermediate pH buffer solution, because the pH of the solution and the ratio of the concentrations of [In$^-$] to [HIn] are found.

In section A.1.2., the ratio of [In$^-$] to [HIn] in the intermediate solution is calculated at each wavelength can be approximated from the measured absorbances of the three buffered solutions with the following simple equation:

Equation 2. $\frac{[In^-]}{[HIn]} = \frac{A_{int.} - A_{low}}{A_{high} - A_{int.}}$

The derivation of Equation 2 is shown below. Let the constant $c = [HIn] + [In^-]$. Thus:

$c \approx [HIn]$ for the low pH solution,
$c \approx [In^-]$ for the high pH solution,
$A_{int}{}^{\lambda} = a_{HIn}{}^{\lambda} b[HIn] + a_{In^-}{}^{\lambda} b[In^-]$,
$A_{lo}{}^{\lambda} \approx a_{HIn}{}^{\lambda} bc$,
$A_{hi}{}^{\lambda} \approx a_{In^-}{}^{\lambda} c$,

$$\Rightarrow \frac{A_{int}{}^{\lambda} - A_{lo}{}^{\lambda}}{A_{hi}{}^{\lambda} - A_{int}{}^{\lambda}} \approx \frac{(a_{HIn}{}^{\lambda} b[HIn] + a_{In^-}{}^{\lambda} b[In^-]) - a_{HIn}{}^{\lambda} bc}{a_{In^-}{}^{\lambda} bc - (a_{HIn}{}^{\lambda} b[HIn] + a_{In^-}{}^{\lambda} b[In^-])} = \frac{b}{b} \times \frac{a_{HIn}{}^{\lambda}([HIn] - c) + a_{In^-}{}^{\lambda}[In^-]}{a_{In^-}{}^{\lambda}(c - [In^-]) - a_{HIn}{}^{\lambda}[HIn]}$$
$$= \frac{a_{HIn}{}^{\lambda}(-[In^-]) + a_{In^-}{}^{\lambda}[In^-]}{a_{In^-}{}^{\lambda}([HIn]) - a_{HIn}{}^{\lambda}[HIn]} = \frac{a_{In^-}{}^{\lambda} - a_{HIn}{}^{\lambda}}{a_{In^-}{}^{\lambda} - a_{HIn}{}^{\lambda}} \times \frac{[In^-]}{[HIn]} = \frac{[In^-]}{[HIn]}$$

(The derivation for Equation 2 uses but does not explain Equation 4 from A.1.3., which states that the sum of the absorbances of two species is equal to the absorbance of a solution containing the both of those species.)

A.1.2. Calculations for pK_a Using Absorbances

An approximation for the ratio of $[In^-]$ to [HIn] (basic and acidic forms of the indicator) in an intermediate pH at a constant wavelength and concentration is Equation 2 (section A.1.1). The pK_a of the solution can be calculated using the pH of the intermediate pH buffer solution and the ratio from Equation 2 using Equation 1 (the Henderson-Hasselbalch Equation). The composite calculation is shown below in Equation 3:

Equation 3. $$pK_a{}^{\lambda} = pH_{int.} + log\left(\frac{[HIn]^{\lambda}}{[In^-]^{\lambda}}\right) = pH_{int.} + log\left(\frac{A_{high}{}^{\lambda} - A_{int.}{}^{\lambda}}{A_{int.}{}^{\lambda} - A_{low}{}^{\lambda}}\right)$$

Equation 3 is used in the sample calculation below to solve for the pK_a at the 440nm wavelength.

$$pK_a{}^{440nm} = 3.95 + log\left(\frac{0.030-0.248}{0.248-0.376}\right) = 4.18$$

A.1.3. Calculation for pKa Using Concentration

The absorbance of a solution with multiple absorbing species at a constant wavelength is the sum of their absorbances. This is expressed in Equation 3 using Beer's law.

Equation 4: $$A_{\lambda} = a_{HIn}{}^{\lambda} b[HIn] + a_{In^-}{}^{\lambda} b[In^-]$$

This expression can be used to find the concentrations of [HIn] and $[In^-]$ if two simultaneous equations at constant concentration and different wavelengths are used. In this case, the optimum wavelengths are chosen as the wavelengths for a set of these equations for the intermediate pH buffer solution.

$$\begin{cases} A_{\lambda} = a_{HIn}{}^{\lambda} b[HIn] + a_{In^-}{}^{\lambda} b[In^-] \\ A_{\lambda} = a_{HIn}{}^{\lambda} b[HIn] + a_{In^-}{}^{\lambda} b[In^-] \end{cases}$$

The coefficients in this system are the slope of the Beer's law plot of the low pH solution at its optimal wavelength ($a_{HIn}{}^{440nm}b$), the slope of the Beer's law plot of the high pH solution at its optimal wavelength ($a_{In^-}{}^{590nm}b$), the absorbance of the high pH solution at the optimum wavelength for the low pH solution ($a_{In^-}{}^{440nm}b$), and the absorbance of the low pH solution at the optimum wavelength for the high pH solution ($a_{HIn}{}^{590nm}b$). The explanation of these values is found in the Discussion section.

The system of equations is solved below using Gauss-Jordan elimination, and the pK_a is found using Equation 3.

$$\left[\begin{array}{cc|c} a_{HIn}{}^{440nm} & a_{In^-}{}^{440nm} & A^{440nm} \\ a_{HIn}{}^{590nm} & a_{In^-}{}^{590nm} & A^{590nm} \end{array}\right] = \left[\begin{array}{cc|c} 0.379 & 0.030 & 0.248 \\ 0.005 & 1.402 & 0.585 \end{array}\right] \rightarrow \left[\begin{array}{cc|c} 1 & 0 & 0.622 \\ 0 & 1 & 0.415 \end{array}\right]$$

$$[In^-] = 0.415M,\ [HIn] = 0.622M,\ \frac{[HIn]}{[In^-]} = 1.50$$

$$pK_a = 3.95 + log(1.50) = 4.13$$

APPENDIX II
COMPUTATION OF STATISTICAL MEASURES OF PRECISION

The calculations for the mean ($\bar{x}$), sample standard deviation (s), standard error (s_m), variance (s^2), and relative standard deviation of the pKa values calculated using method 1 are shown below. Because the data points are roughly spread uniformly over a small range and not heavily skewed, the mean will be used to represent the center and the standard deviation will be used to estimate the range (as opposed to the median and IQR). The data analyzed here is from Appendix 3 Table 8.

The data collected at the 375nm wavelength is omitted from the data because its value is undefined (the calculation takes the logarithm of a negative number).

$$n = 21$$

$$\bar{x} = \frac{1}{n}\sum_{k=1}^{n}(calculated\ pKa)_k = 4.10$$

$$s = \sqrt{\frac{1}{n-1}\sum_{k=1}^{n}((calculated\ pKa)_k - (mean\ pKa))^2} = 0.168$$

$$s_m = \frac{s}{\sqrt{n}} = 0.0367$$

$$s^2 = 0.0283$$

$$rel.\ std.\ dev.\ (ppt) = 1000 \times \frac{s}{\bar{x}} = 41.0$$

A Q test for outliers is performed below at a 90% confidence level.

$$range = 4.25 - 3.38 = 0.88$$

$$90\%\ confidence\ Q_{crit_{21}} = 0.295$$

$$Q_{4.2510} = \frac{|4.25-4.18|}{0.88} = 0.079 < Q_{crit}$$

$$Q_{3.3760} = \frac{|3.38-4.06|}{0.88} = 0.78 > Q_{crit}$$

The Q-value for the maximum does not exceed Q_{crit}, so it is not statistically justified to remove it. However, the Q-value for the minimum value, 3.38, is far above Q_{crit}, so it is statistically justified to remove this data point. Besides the impossible data point removed earlier, this is the only questionable data point that lies far from the rest of the data; the potential causes of error leading to this are discussed in the discussion section.

Now omitting both the indeterminate data point and this statistical outlier, the statistics are re-calculated. These statistical values are used in the discussion and conclusion.

$n = 20$

$\bar{x} = \frac{1}{n} \sum_{k=1}^{n} (calculated\ pKa)_k = 4.13$

$s = \sqrt{\frac{1}{n-1} \sum_{k=1}^{n} ((calculated\ pKa)_k - (mean\ pKa))^2} = 0.0477$

$s_m = \frac{s}{\sqrt{n}} = 0.0107$

$s^2 = 00228$

$rel.\ std.\ dev.\ (ppt) = 1000 \times \frac{s}{\bar{x}} = 11.5$

The new standard deviation is much smaller after the removal of the single large outlier. The calculation of a 90% confidence interval for the standardized is shown below.

90% *confidence level* $t_{n-1} = t_{19} = 1.729$

uncertainty $(u) = t_{n-1} \times \frac{s}{\sqrt{n}} = 0.0185$

90% *confidence interval* $= 4.13 \pm 0.02$

The percent error of the pK_a is calculated using 4.10 as the literature value. The percent errors of the mean of method 1 and method 2 is shown below.

$\%\ Error_1 = \frac{|4.13=4.10|}{4.10} \times 100\% = 0.8\%$

$\%\ Error_2 = \frac{|4.13=4.10|}{4.10} \times 100\% = 0.6\%$

(The difference in the percent errors is due to the fact that the calculated pK_a values are displayed rounded to two decimal places and appear the same but actually vary slightly).

APPENDIX III
pH COLOR CHANGES AND VISIBLE ABSORPTION SPECTRA DATA

The qualitative recorded color of a solution containing the indicator at 0.1 pH unit intervals is shown below. The solution initially consisted mostly of acetic acid with a few drops of indicator, and sodium hydroxide was added until the color change was thought to be completed.

Table 7: pH vs. Color Changes as NaOH is Added

pH	Color	pH	Color	pH	Color
2.3	yellow	3.3	dark yellow-green	4.3	violet
2.4	yellow	3.4	light green	4.4	violet
2.5	yellow	3.5	green	4.5	violet
2.6	yellow	3.6	dark yellow green	4.6	violet
2.7	yellow	3.7	very dark yellow green	4.7	violet
2.8	yellow	3.8	dark red-violet	4.8	violet
2.9	yellow	3.9	dark violet	4.9	violet
3.0	yellow	4.0	dark violet	5.0	violet
3.1	slightly-dark yellow	4.1	dark violet	5.1	violet
3.2	dark yellow-green	4.2	violet		

The absorbance versus wavelength for each of the three buffered indicator solutions is shown below in Table 8. Also shown in the Table 8 is the pK_a value calculated by Equation 3 (section A.1.2) for each wavelength.

Table 8: Absorbance for each Buffered Solution and Calculated pK_a vs. Wavelength

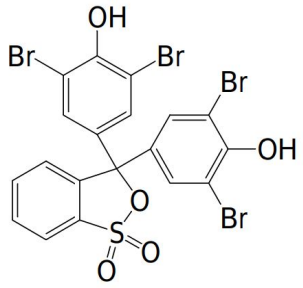
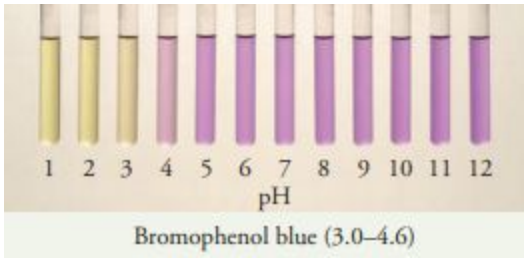
Wavelength (nm)	Absorbance			Calculated pK_a
	Low pH	Medium pH	High pH	
350	0.106	0.091	0.087	3.38
375	0.148	0.142	0.148	—*
400	0.255	0.187	0.090	4.10
425	0.367	0.233	0.026	4.14
430	0.369	0.240	0.029	4.16
435	0.374	0.244	0.027	4.17
440	0.376	0.248	0.030	4.18
445	0.373	0.248	0.036	4.18
450	0.371	0.246	0.044	4.16
475	0.250	0.189	0.086	4.18
500	0.125	0.146	0.181	4.17
525	0.042	0.161	0.351	4.15
550	0.010	0.264	0.650	4.13
575	0.006	0.434	1.076	4.13
580	0.004	0.490	1.207	4.12
585	0.005	0.547	1.335	4.11
590	0.005	0.585	1.409	4.10
595	0.005	0.581	1.378	4.09
600	0.005	0.521	1.213	4.08
605	0.004	0.414	0.949	4.07
610	0.004	0.303	0.688	4.06
625	0.002	0.08	0.185	4.07
650	0.001	0.006	0.016	4.25

* The pKa value is indeterminate because the calculation involved calculating the logarithm of a negative value; see calculations section for more details.

APPENDIX IV
INDICATOR LITERATURE VALUES

In Table 9, a list of literature values for the conjectured unknown indicator, bromophenol blue (see Discussion), are displayed. If multiple values are found, the value used is starred.

Table 9. Indicator Literature Values

Common name	Bromophenol Blue
IUPAC name	2,6-dibromo-4-[3-(3,5-dibromo-4-hydroxyphenyl)-1,1-dioxo-2,1$l^{6}-benzoxathiol-3-yl]phenol; *National Center for Biotechnology Information* (6)
Structural formula	 *CRC Handbook of Chemistry and Physics* (5)
Molecular formula	$C_{19}H_{10}Br_4O_5S$; *CRC Handbook of Chemistry and Physics* (5)
Molecular weight	669.960g/mol; *CRC Handbook of Chemistry and Physics* (5)
pK_a	4.10*; *CRC Handbook of Chemistry and Physics* (5) 4.1; Kulichenko (3) 4.0; O'Neil (4)
Color changes	Y-B; *CRC Handbook of Chemistry and Physics* (5)  Skoog et al. (2)
Effective pH range	3.0-4.6; Skoog et al. (2), *CRC Handbook of Chemistry and Physics* (5)