Measurement of the fractional abundance diagram of thymol blue using spectrophotometry

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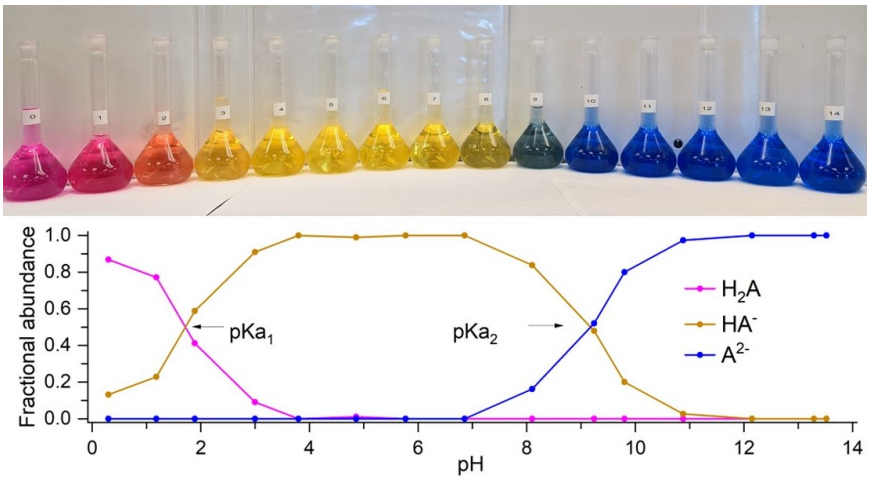
Fractional abundance
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
pH
pKa1
pKa2
H2A
HA-
A2-

ABSTRACT

In both general chemistry and analytical chemistry courses, students are introduced to the concept of predominant species in solution when discussing acid/base chemistry. Fractional abundance diagrams are often used to illustrate the concept and predict the relative abundance of species in solution. Herein, we describe a laboratory experiment for an undergraduate analytical chemistry course, in which students directly measure the fractional abundance diagram and compare it to the diagram calculated using equations they derive in lectures. In this laboratory experiment, students prepare solutions of fixed pH in the pH range 0-14 and use them to measure the fractional abundance diagram of thymol blue with UV-Vis spectrophotometry. After collecting the absorbance spectra of all the solutions, students perform a series of data processing steps to derive the fractional composition diagram of thymol blue from the experimental data and compare it to the calculated diagram. Finally, students use the fractional composition diagram to corroborate the expected pH ranges, in which the three forms of thymol blue are predominant species. This experiment provides students with a hands-on experience with buffer preparation, enables a straightforward measurement of the fractional abundance of different forms of a diprotic acid, and helps them visualize the concept of predominant species in solution.

INTRODUCTION

Acid/base chemistry is a major topic in both general and analytical chemistry courses. Several analytical determinations use acid/base properties of an analyte to separate or quantify it in a complex mixture. A substantial effort has been dedicated towards the investigation of students' understanding of acid/base chemistry[1–5] and the implementation of activities[6–8] and experiments[9,10] that promote the conceptualization of this class of reactions in undergraduate chemistry courses. For students to adequately design an experiment or perform pH calculations, they must accurately predict whether a compound is present in its acidic, basic, or intermediate form in solution at a given pH.[11–14]

The concept of predominant species in solution is usually taught using fractional composition diagrams, in which the relative abundance of each species is plotted as a function of pH. Despite the importance of this topic, undergraduate chemistry laboratory experiments focused on measuring fractional composition diagrams are rare. A relatively straightforward approach to perform this measurement relies on spectrophotometry. Thymol blue is a pH indicator that is well-suited for this kind of laboratory experiment. Indeed, thymol blue is one of the few pH indicators that exists in three forms, all of which absorb light in the visible region at distinct absorbance wavelengths that do not overlap significantly.[15] As a result, thymol blue solutions exhibit dramatic color changes as a function of pH, which is both visually attractive for students and easy to measure in an experiment. Recently, Yimkosol., *et.al.,* reported an online undergraduate experiment for determining the two pK_a values of thymol blue using the RGB values obtained from digital images of thymol blue solutions at different pH.[16] Herein, we describe a different undergraduate laboratory experiment, in which the distinct colors of thymol blue solutions are used to directly measure the fractional abundance diagram for this system with UV-Vis spectrophotometry. Specifically, students used the absorbance spectra of 15 solutions, each at integer pH values in the pH range 0-14 to determine the relative abundance of different protonation forms of thymol blue. Because this experiment requires the preparation of solutions with fixed pH values, students also prepared several buffer solutions, which gave them an opportunity to master this important skill.[17,18] This laboratory experiment helps students integrate concepts from acid/base equilibria, acid/base buffers, and spectrophotometry to experimentally visualize the regions where different forms of a diprotic acid are predominant like they learn about in lectures.

DESCRIPTION OF THE EXPERIMENT

Participants. This experiment was implemented as a 3-hour laboratory activity in the Quantitative Analysis course with 102 students who were mainly 3rd and 4th year undergraduate students. Each laboratory was

organized into sections of 21 students with seven stations available to perform the experiments with most students working in groups of three.

Pre-lab requirements. Prior to attending the lab, students are provided with the lab protocol and narrated PowerPoint slides (both included in the SI), in which a teaching assistant provides an overview of the lab. Once students read and listened to the narrated PowerPoint slides, they take a pre-lab quiz (provided in the SI). In the pre-lab quiz, students are asked five questions aimed to test their understanding of the experiment. These pre-lab assignments ensure that students are familiar with the experiment before coming to the lab.

Experiment design. The goal of the activity was to measure the fractional abundance of thymol blue using spectrophotometry. Thymol blue was selected as an analyte because it is a diprotic acid and an acid/base indicator. The most acidic form of thymol blue (H_2A) is pink, the deprotonated form (HA^-) is yellow, and the basic form (A^{2-}) is blue. Thymol blue solutions of different pH have different colors that may be readily distinguished using spectrophotometry.[15,19] To cover the entire pH range, a total of 15 solutions of fixed pH were prepared. Since the preparation of 15 solutions is a demanding task for a 3 hour long laboratory experiment, we asked students to divide the solution preparation work as follows. First, each group of 3 students prepared two stock solutions of fixed pH that they shared with the rest of the lab section. Then, students used these solutions and a stock solution of thymol blue provided to them to prepare 15 solutions of thymol blue of different pH. Students used these solutions for the spectrophotometric measurement of the fractional abundance of thymol blue. Students had one week to complete the lab report, in which they described the background of the experiment, summarized the experimental procedure, showed the main results of the measurements, and discussed their results. In the results section of the lab report, students compared the calculated and experimental fractional abundance diagrams across the entire pH range and discussed their observations in the context of predominant species in a given pH interval.

LEARNING OUTCOMES

By performing this project, students will:

1) Design and execute an experimental methodology to prepare a solution of fixed pH.
2) Reflect on the changes observed in the absorbance spectra of thymol blue as the pH changes.
3) Identify the wavelength of maximum absorbance for each of the three thymol blue species.
4) Execute a data processing protocol to convert the experimental absorbances into the fractional composition diagrams of thymol blue species.

5) Calculate the theoretical fractional abundance diagrams of the thymol blue species and compare with the measured ones.
6) Use the fractional abundance diagrams to determine the range of pH at which each species of thymol blue is predominant.

EXPERIMENTAL SECTION

Preparation of stock solutions of fixed pH. A total of 15 solutions of fixed pH were used to cover the entire pH range. The solutions were prepared in 500.0 mL volumetric flasks with a total concentration of acid and base equal to 0.05 M. The acids and bases used for the preparation of each solution are listed in Table 1. A complete list of chemicals and preparation details for all the solutions are provided in the SI for instructors.

Table 1. Chemical composition of the fifteen fixed pH solutions.

Solution pH	Composition
0	HCl 1 M
1	HCl 0.1 M
2	HCl 0.01 M + KCl 0.04 M (to keep the ionic strength = 0.05 M)
3	Citric acid 0.027 M + sodium citrate 0.023 M
4	Acetic acid 0.042 M + sodium acetate 0.008 M
5	Acetic acid 0.018 M + sodium acetate 0.032 M
6	Sodium phosphate monobasic 0.047 M + sodium phosphate dibasic 0.003 M.
7	Sodium phosphate monobasic 0.031 M + sodium phosphate dibasic 0.019 M.
8	Tris HCl 0.028 M + Tris base 0.022 M
9	Tris HCl 0.006 M + Tris base 0.044 M.
10	Ammonium chloride 0.008 M + ammonia 0.042 M.
11	Sodium bicarbonate 0.009 M + sodium carbonate 0.041 M.
12	Sodium phosphate dibasic 0.038 M + sodium phosphate 0.012 M.
13	NaOH 0.1 M
14	NaOH 1 M.

For each solution, the chemicals were weighed, mixed, and dissolved in DI water to a volume of less than 500 mL, which was sufficient for the entire lab section. Students used a previously calibrated pH electrode (Vernier) to measure the pH of each solution they prepared. If the pH of the solution was not within 0.2 units of the target value, they added small volumes of 2 M HCl or 2 M NaOH to bring the pH closer to the desired value. Unless sample preparation was not performed accurately, the measured pH of the solution should be close to the expected value. Finally, students transferred quantitatively each solution they prepared into a 500.0 mL volumetric flask and diluted to the mark with DI water. The 15 solutions were then placed under a shared hood for all the students to use.

Spectrophotometric measurement. To measure the fractional abundance diagram, students used thymol blue as an analyte. For this measurement, students prepared 15 solutions of 2×10^{-5} M thymol blue, one per pH value. These solutions were prepared by diluting an aliquot of a stock thymol blue solution (5×10^{-4} M) into the solutions of fixed pH prepared in the previous step. All the 15 solutions were analyzed using UV-Vis spectrophotometry, independently. In these experiments, an aliquot was placed in a 1.00 cm plastic cuvette and its full absorbance spectrum was recorded using a Vernier Fluorescence/UV-Vis spectrophotometer.

HAZARDS

Students are required to wear appropriate lab clothing including splash googles and gloves during the entire lab session. HCl, citric acid, acetic acid, NH_3, and NaOH are corrosive.

RESULTS AND DISCUSSION

Preparation of stock solutions of fixed pH. In the laboratory, students first calculated the amounts of chemicals they need to prepare the fixed pH solutions. At the start of the session, students were given a list of all the chemicals available to them in the lab. Students were also given a table of the suggested acid/base pairs to prepare each of the solutions along with their pK_a values. To calculate the amount of acid and/or base required to prepare each solution assigned to them, students considered two general cases:

1) Solutions of pH 0, 1, 13, and 14 are prepared from a strong acid or strong base. This means that the concentration of the acid or base can be calculated directly from the definition of pH or pOH.
2) A solution at pH = 2 presents a special case, in which KCl is added to the strong acid to ensure constant ionic strength of the resulting solution.
3) For solutions that are prepared using weak acid/base pairs, the concentrations of the acid and base are calculated using equations 1 and 2:

$$C_T = C_A + C_B \qquad (1)$$

$$pH = pKa + log\frac{C_B}{C_A} \qquad (2)$$

where C_A and C_B are molar concentrations of the acid and base, respectively, and C_T is the total molar concentration. For polyprotic systems, such as citric acid/sodium citrate, phosphates and carbonates, students selected the pK_a value of the acid/base pair they used to prepare the solution and treated it as a monoprotic system. Because pK_a values of subsequent deprotonations are relatively far from each other, this assumption will not result in a significative error in the pH estimation.

Students calculated the concentrations of acid and/or base necessary to prepare the solutions assigned to them, and the amount (mass or volume) of the compound they needed to measure. This step required students to consider the physical state and purity of the chemicals available in the lab. Before preparing the solutions, students showed their work to their TA to corroborate their results.

Shown in Box 1 are example calculations and notes that a student made to determine how to prepare the pH 9 and 13 solutions. The student used equations 1 and 2 to find the concentrations of the acid/base pair in the buffer 9 solution and used the pOH definition to calculate the concentration of NaOH in the pH 13 solution.

Solution to prepare	Acid	Base	pK_a
9	TRIS HCl	TRIS base	8.10
13	---	NaOH	---

pH 9 solution

$$pH = pKa + log\frac{C_B}{C_A}$$

$$9 = 8.10 + log\frac{C_B}{0.05 - C_B} \rightarrow 10^9 = 10^{8.10} \times \frac{C_B}{0.05 - C_B}$$

$$\frac{C_B}{0.05 - C_B} = 7.9433 \rightarrow C_B = 0.0444\ M, C_A = 0.05 - C_B = 0.0056\ M$$

$$\boldsymbol{m_B} = 0.0444\ M \times 0.5\ L \times 121.1\frac{g}{mol} = \mathbf{2.6890}\ \boldsymbol{g}$$

$$\boldsymbol{m_A} = 0.0056\ M \times 0.5\ L \times 157.6\frac{g}{mol} = \mathbf{0.4406}\ \boldsymbol{g}$$

Experimentally, 0.4798 g of TRIS HCl and 2.6907 g of TRIS base were dissolved in a beaker. Solution transferred into a 500 mL volumetric flask; the measured pH was 9.25. Rinse the beaker 3 times with DI water, which is then transferred into the flask. Filled the flask to the mark with DI water.

pH 13 solution

Mass of NaOH = $0.1\ M \times 0.5\ L \times 40\frac{g}{mol} = \mathbf{2}\ \boldsymbol{g}$

NaOH buffer pH = 12.70. 2.0055 g NaOH solid was measured and placed in a beaker. Dissolve the solid using DI water and transferred solution into a 500 mL volumetric flask. Rinsed the beaker three times with DI water, which is then transferred into the volumetric flask. Fill the flask to the mark and inverted it for mixing.

Box 1. Example of the calculations and notes made by a student regarding the preparation of solutions of fixed pH.

Spectrophotometric measurement. Figure 2 shows the 15 thymol blue solutions prepared by a group of students. The fully protonated form of thymol blue (H_2A) is predominant at pH<2 and forms pink colored solutions. The partially protonated form of thymol blue (HA^-) is predominant in the pH range of 2-8 and gives yellow solutions. Finally, the fully deprotonated form of thymol blue (A^{2-}) forms blue colored solutions at pH above 8.

Figure 2. Solutions of 2×10^{-5} M thymol blue at different pH (increasing pH from left to right).

Students measured the UV-Vis absorbance spectra of the 15 thymol blue solutions at different pH values. Representative examples of the absorbance spectra obtained by students are shown in Figure 3. Each thymol blue species has a distinct absorbance maximum: 545 nm for H_2A, 430 nm for HA^-, and at 600 nm for A^{2-}. Students also noted the presence of an isosbestic point at 480 nm indicative of a two-component equilibrium system they learned about in lectures. To succeed in this experiment, students should be mindful that the concentration of thymol blue remains constant among the 15 different solutions; otherwise there will be an error in the absorbance measurement. Rinsing the cuvette twice with DI water and once with the solution prepared for analysis before the measurement ensures more accurate readings.

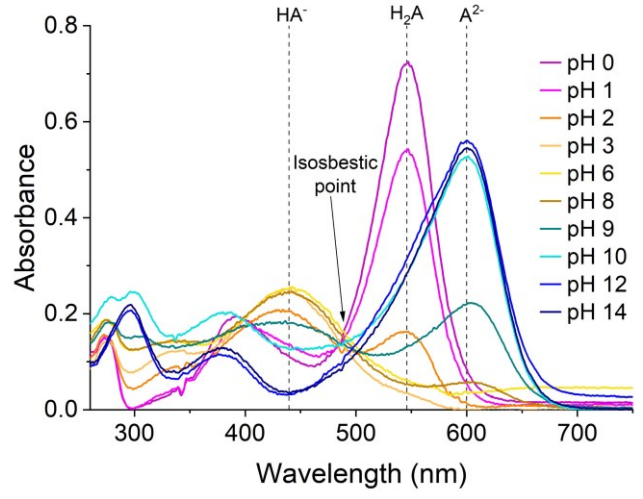

Figure 3. Absorbance spectra of thymol blue at different values of pH. The wavelengths of maximum absorbance for H_2A, HA^-, A^{2-} are at 545, 430 and 600 nm, respectively. The isosbestic point is observed at 480 nm.

Construction of fractional abundance diagrams. For their lab report, students generated the theoretical and experimental fractional composition diagrams for each of the thymol blue species using Microsoft

Excel. Theoretical fractional abundance curves were calculated using equations 3-5 in 0.1 pH increments using $pK_{a1} = 1.7$ and $pK_{a2} = 8.9$.[15]

$$\alpha_{H_2A} = \frac{[H^+]^2}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (3)$$

$$\alpha_{HA^-} = \frac{K_{a1}[H^+]}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (4)$$

$$\alpha_{HA^{2-}} = \frac{K_{a1}K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (5)$$

Figure 4 shows the results obtained in each step of data analysis focused on generating the experimental fractional abundance diagrams from measured absorbances . The resulting diagrams are shown in Figure 5. The experimental fractional composition diagrams were calculated as follows.

1. For each solution, students created a table containing absorbances at the three wavelengths of maximum absorbance of 545, 430, and 600 nm used to estimate the concentrations of H_2A, HA^-, and A^{2-}, respectively, in solution. A plot of the measured absorbance as a function of pH is shown in Figure 4a.
2. At each wavelength, the lowest absorbance was subtracted from all other absorbances to eliminate the contribution from other species present in solution that absorb at a specific wavelength. The result of this operation is referred to as "corrected absorbance" and it shown in Figure 4b.
3. At each wavelength (i.e. for each species), students identified the highest corrected absorbance and divided all the corrected absorbances by this value. These results are referred to as "normalized absorbances". The result of this calculation is shown in Figure 4c. This step is necessary to normalize the absorbance to the maximum observed absorbance and obtain fractional abundances that are independent of the molar absorptivity.
4. Next, students inspected the values of normalized absorbances and eliminated artifacts that originate from interferences. For example, the absorbance at 545 nm attributed to H_2A initially decreases with an increase in pH but at high pH it increases again. This behavior can be explained by examining the absorbance spectra shown in Figure 3. The apparent increase in absorbance of H_2A at high pH results from the growth of a broad absorbance peak of A^{2-} at 600 nm. Students recognize the presence of interferent absorbance values and replace the normalized absorbance of H_2A at high pH with zeros. They recognize that data obtained for each species must be treated differently. To correct for other interferences, students use the known pK_a values of thymol blue to predict what are the principal species of thymol blue in solution in each pH range. For H_2A, normalized absorbances at pH higher than 4 are replaced with zeros since less than 1% of H_2A is

present in this range. For HA^-, absorbances at pH higher than 11 are replaced with zeros. For A^{2-}, absorbances at pH lower or equal to 7 are replaced with zeros. The result of this processing step is shown in Figure 4d. An alternative approach would involve solving for the individual absorbances of each species using the mixture analysis procedure they learned about in lectures. Because these calculations are tedious, we did not incorporate them into the data analysis procedure.

5. The experimental fractional abundances are calculated by adding the absorbances of the three species at each pH value. This value represents the total amount of thymol blue. The fractional abundances are calculated by dividing the absorbance of each species at a given pH by the total absorbance at that pH. The result of all the processing steps is shown in Figure 5.

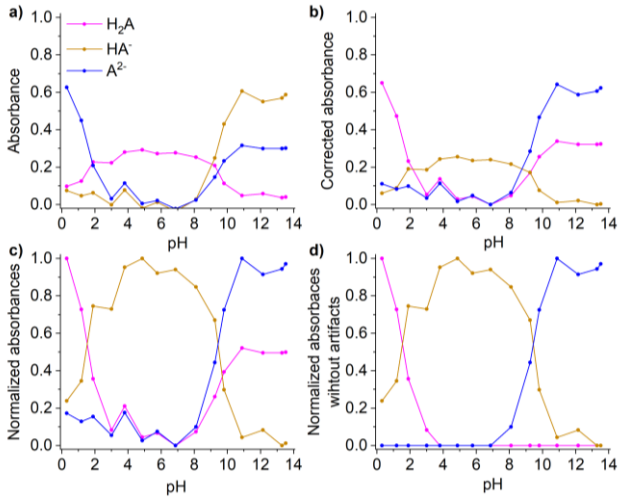

Figure 4. Visualization of the experimental results after each data processing step. a) Experimentally measured absorbances, b) corrected absorbance step (step 2), c) normalized absorbances (step 3), and d) normalized absorbances without artifacts (step 4).

Students observed a good correspondence between the experimental and theoretical fractional composition diagrams as shown in Figure 5. As described previously, the observed deviation from the expected theoretical trend is attributed to the experimental errors in solution preparation. Examples of experimental errors include variations in the concentration of thymol blue in the analyzed solutions and solution contamination when placed in the cuvette. These deviations are more pronounced in the pH ranges close to the pK_a values since the relative abundance of species change dramatically in these regions. The agreement between the experimental and theoretical diagrams can also be improved by using more robust mathematical multicomponent analysis to determine the concentration of each species.[15,20–22] The advantage of the methodology described above is in its simplicity and accessibility for students regardless of their mathematical background.

Students used their experimental results to determine that H_2A is the thymol blue predominant species when $pH < pK_{a1}$, HA^- is when $pK_{a1} < pH < pK_{a2}$, and A^{2-} is the major species when $pK_{a2} < pH$.

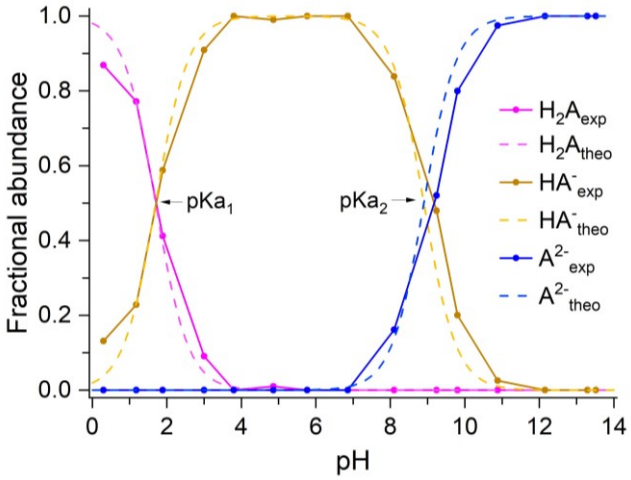

Figure 5. Experimental (solid lines with markers) and theoretical (dashed lines) fractional composition diagrams of the thymol blue species.

EVALUATION OF LEARNING OBJECTIVES

During the laboratory experiment, the TA worked with the students to corroborate that they correctly performed the calculations to prepare the solutions of fixed pH. Most of the students succeeded to get the right answer in the first attempt and the rest of them did it on the second attempt after some guidance from the TA. Common sources of errors in the calculation include dimensional analysis and confusing the acid with the base in the Henderson-Hasselbalch equation.

Students had one week to prepare a full lab report in which they showed the overlay between the experimental and theoretical fractional composition diagrams, labeled the pK_a's, and described the pH intervals in which different forms of thymol blue are predominant. The rubric used to evaluate the lab reports is provided in the SI. Based on the lab reports submitted by students, more than 95% of them were able to create fractional abundance diagrams like the one shown in Figure 4. The same number of students successfully identified the pH range in which each species is predominant based on the inspection of the fractional abundance diagrams. As described earlier, students recognized the importance of accurate solution preparation on the quality of the fractional abundance diagrams.

Students also answered an online post-lab quiz to further assess their understanding of the concepts and calculations related to this lab. Table 1 shows example questions that students answered in the post-quiz. The results of the quiz indicate that most of the students in this class were familiar with calculations related the effect of dilution on the pH of a buffer. It was surprising to see that at least 25% of the class did not

correctly answer the question regarding buffer preparation since during the laboratory this percentage was not as high. It is likely that the errors in the calculation observed in the laboratory were repeated in the post-lab quiz. These errors are more likely to arise in a one-attempt only test as opposed to in lab where students can get feedback from their peers and instructor.

Topic	Main text of the question			
Buffer preparation (calculated)	A buffer solution is prepared in the lab by mixing a weak acid, HA (pK_a = 3.46), with a salt of its conjugate base, KA. What mass (in grams) of HA (MW = 107.0 g/mol) must be weighed to prepare 0.5 L of this buffer with a total concentration of 0.045 M and pH = 2.60? **Answer = 2.1155 g.**			
	Correct responses	74.5%	Incorrect responses	25.5%
Buffer dilution (calculated)	A buffer was prepared in 350 mL and the pH adjusted to 5.85. The solution was diluted to a final volume of 500.0 mL with DI water. What is the pH of the buffer after dilution to 500.0 mL? **Answer = 5.85**			
	Correct responses	81.4%	Incorrect responses	18.6%
Properties of thymol blue (multiple choice)	Which statement is TRUE regarding Thymol blue: A) The color transition at low pH is from green to purple., B) It is not a pH indicator, C) It is a triprotic acid, D) It has two pK_a's. **Answer = D.**			
	Correct responses	97.1%	Incorrect responses	2.9%
Data processing (multiple choice)	During the data processing, why was it necessary to create the column "corrected absorbance"? A) To find the pK_a, B) Eliminate residual absorbance due to other species that absorb at a particular wavelength, C) Eliminate the absorbance of the species of interest, D) Normalize the absorbances. **Answer = B.**			
	Correct responses	90.2%	Incorrect responses	9.8%

CONCLUSIONS

We describe the implementation of a laboratory experiment, in which students measure the fractional composition diagram of thymol blue using UV-Vis spectrophotometry. This involves the preparation of 15 solutions of fixed pH in the pH 0-14 range, which students carried out during the lab. Students calculated

the amounts of compounds required to prepare the solutions they were assigned to. To perform the calculations, students used chemical reasoning to determine which mathematical equation they should use based on the characteristics of the acid/base pair. In the lab, students measured the absorbance spectra of 15 thymol blue solutions of fixed pH. Students identified the wavelengths of maximum absorbance of thymol blue species to be 545, 430 and 600 nm for H_2A, HA^-, and A^{2-}, respectively. Absorbances at these wavelengths were used to calculate the fractional composition diagram of thymol blue. Meanwhile, the predicted fractional composition diagram was calculated using standard equations introduced in lectures. Students observed a good correspondence between the measured and calculated diagrams and concluded based on their experimental data that H_2A is the predominant species in solution at $pH<pKa_1$, HA^- is predominant at $pKa_1 < pH < pKa_2$, and A^{2-} is predominant at $pH > pKa_2$. The post-lab quiz indicates that students qualitatively understood the reasoning behind using thymol blue as an analyte for this experiment, learned how to prepare solutions of a fixed pH, and understood the data processing procedure. Further implementations of this activity will benefit from giving more guidance to students and strengthening their confidence on how to perform calculations to prepare solutions of a fixed pH.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

SUPPORTING INFORMATION

Prep-lab notes, lab protocol for students, narrated power point with key experimental details, rubric to evaluate lab report.

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