Spectroscopic and Potentiometric Investigation of a Diprotic Acid: An Experimental Approach to Understanding Alpha Functions

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Abstract: A laboratory experiment suitable for second- or higher-year chemistry students uses thymol blue, an organic dye, for investigating the fraction of each colored species present as a function of pH. Visible color changes in the vivid, highly absorbing dye permit students to “see” relative amounts of acid, base, and ampholyte species during the titration, while potentiometric measurement of pH and spectroscopic determination of concentrations permit quantitation of the species present. A standard Beer’s law calibration curve is constructed for known concentrations of the pure forms of each species at a pH where that species is present as nearly 100% of the total analytical concentration of the dye. Using the molar absorptivities of the three species computed from the calibration curves, simultaneous determination of concentration by species is performed on a set of solutions having constant total concentration of dye such that each solution represents one point in the range pH 0 to pH 13. A mathematical software application is used to solve the simultaneous equations and determine the fraction of each species present. Students then use a spreadsheet application to plot the fractions as functions of pH (the alpha functions). The significance of the isosbestic points in the spectra is also explored.

Introduction

The ionization of indicator dyes has been studied intensively by Kolthoff, beginning early in this century [1–14]. Thymol blue (TB) indicator dye has been studied in detail recently, as a spectroscopic pH indicator in complex matrices such as seawater [15] and wastewaters. Spectroscopic pH determination has been shown to be far more precise and accurate than potentiometric pH measurements when a well-characterized primary standard reference material is used [16–18]. We present an experiment used in our first analytical chemistry course, usually taken in the sophomore year. This experiment explores the acid-base ionization behavior of thymol blue, both spectroscopically and potentiometrically, introducing students to spectroscopic pH determination while addressing more traditional pedagogical goals for the first analytical laboratory. The experiment is also suitable for a special project in higher-level courses.

The sophomore-level analytical course traditionally deals with fundamental concepts of equilibrium chemistry, and it lays a foundation for advanced equilibrium topics covered in upper division coursework. Recent innovations in analytical curriculum development reflect changes in the analytical work place. Instrumentation is now frequently introduced at this level of instruction and includes the pH electrode and spectrophotometers. Data-reduction techniques often include the use of spreadsheets and mathematics software applications, such as MathCad and Maple. Simultaneous determination of multiple unknowns is increasingly expected at this level. In addition, there is a trend toward emphasis on teamwork in solving more complex analytical problems. Students working in teams and sharing data can explore several different aspects of the same question. This experiment addresses all of these goals and provides discovery-based learning for several concepts that are historically difficult for students to understand, such as why the equivalence point of some weak acids is not observed in the titration curve of a mixture of weak and strong acids, how to interpret α-function plots, and what is the significance of an isosbestic point [19].

By the sophomore year, students are usually familiar with a weak-acid versus strong-base titration. The traditional quantitative experiment, determination of a potassium hydrogen phthalate (KHP) unknown using phenolphthalein indicator, is therefore redundant except for the greater precision and accuracy required in the second-year laboratory course. An experiment that addresses the traditional goals of the course while exposing students to more advanced concepts and applications of equilibrium chemistry would have greater pedagogical value and be more interesting to the students. Furthermore, student interest in environmental chemistry can be promoted by pointing out the applications of this particular experiment to seawater and wastewater analyses, as referenced above.

In this laboratory experiment, the acid dissociation equilibria of a weak diprotic organic dye are examined in some detail by spectroscopy and potentiometry. The fully protonated, ampholytic, and fully deprotonated forms of thymol blue are all highly absorbing and each form has a unique color (Figure 1). This allows visual inspection of solutions, which provides some intuitive grasp of the equilibrium processes occurring. Students find the brilliant colors of the various forms aesthetically pleasing, as well.

The α-functions for the three species of the diprotic system at different pHs are determined empirically from visible spectroscopic data and plotted against pH. Students work as a team to prepare a series of dilutions and generate a Beer’s law calibration curve for each of the three species at each of three wavelengths, to extract molar absorptivities from spectroscopic data, and to explore the significance of an isosbestic point. The full titration curve of a known solution is generated and plotted revealing the behavior of the “not-so-weak” first deprotonation step (equivalence point is not observed). The second equivalence point is determined by numerical analysis of the titration data. Because the first

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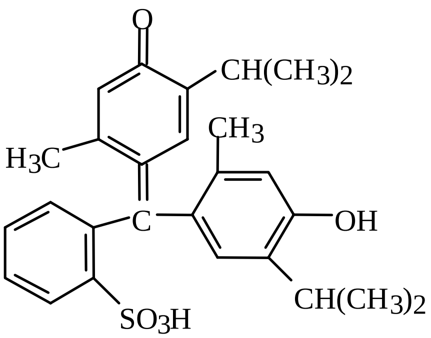


Figure 1. Thymol Blue, fully protonated form.

Table 1. Dilutions of TB solution (Stock = 2.23 mg (95%) TB in 100 mL). TB stock is first diluted 1:5 to prepare a solution having 0.446 mg TB per 100 mL

Volume of 1:5 diluted TB in 10 mL vol. flask	Concentration (mol L^{-1})
Red form; pH = 0.0	
0.5 mL	4.336×10^{-6}
1.0	8.672×10^{-6}
1.5	1.301×10^{-5}
2.0	1.734×10^{-5}
2.5	2.168×10^{-5}
Yellow form; pH ~6.0	
0.5 mL	4.336×10^{-6}
1.0	8.672×10^{-6}
2.0	1.734×10^{-5}
4.0	3.47×10^{-5}
6.0	5.20×10^{-5}
8.0	6.94×10^{-5}
Blue form; pH = 12.8	
0.5 mL	4.336×10^{-6}
1.0	8.672×10^{-6}
1.5	1.301×10^{-5}
2.0	1.734×10^{-5}
2.5	2.168×10^{-5}

dissociation step is not-so-weak ($K_a \sim 10^{-2}$) students gain first hand experience with the problem of the "invisible" equivalence point for such a system.

A set of equal concentration solutions is prepared through the pH range 0 to 13 and the visible spectrum recorded for each. Overlay of these curves reveals several isosbestic points for each transition. A mathematical application is used to solve simultaneous equations of the absorbance data and determine concentration of each species at each pH. Students generate curves for the fraction of each species with respect to pH. These curves are labeled with the color observed at each pH and analyzed for a qualitative understanding of the equilibria occurring and the relative proportions of each species in various pH ranges. The two acid-dissociation constants are determined from the α-function plots, and the pH of the ampholyte (only) and base (only) species is compared with the end point of the titration. Student understanding of these important curves is greatly increased through the exercise of generating the plots from empirical data. Students are asked to compare experimental and theoretical α-functions. The experiment can be exploited for more traditional quantitative analysis by having the students determine an unknown, prepared by the instructor.

On a practical level, the procedure is very demanding and students must observe strict quantitative protocols in order to obtain high-quality data. This experiment uses a minimum amount of ordinary reagents and laboratory glassware and is inexpensive and relatively nonhazardous. Students should be briefed on the correct handling of the strong mineral acid and base. The waste from the dye solutions should be disposed of in accordance with all state and local regulations. (Many localities permit disposal of dye solutions in the sewage system, after decolorization with hypochlorite bleach.)

Experimental

Chemicals Required. Water-soluble sodium form of thymol blue (TB), 95 % purity, (ACS analytical reagent grade) can be obtained from Aldrich or from Matheson, Coleman, and Bell. Students need concentrated hydrochloric acid and 6 M sodium hydroxide solutions, as well as dilute (1 M) and very dilute (~1 mM) solutions of each of these reagents for fine tuning the pH of the dye solutions.

Instrumentation

Spectroscopy. A scanning, recording spectrophotometer is optimal; however, the experiment can be performed using a single-beam spectrometer such as the Bausch and Lomb Spec 20. If this instrument is chosen, students should not be expected to examine the isobestic points. Data reported here were recorded using an HP model 8453 photodiode array spectrophotometer. Inexpensive glass or plastic cuvettes can be used for the spectral region in this experiment.

pH Electrode and Electrometer. Any student-model electrometer and glass pH electrode could be used, in principle; however, better results will be obtained with a high-impedance electrometer and a full-range lithium glass electrode.

Equipment and Supplies

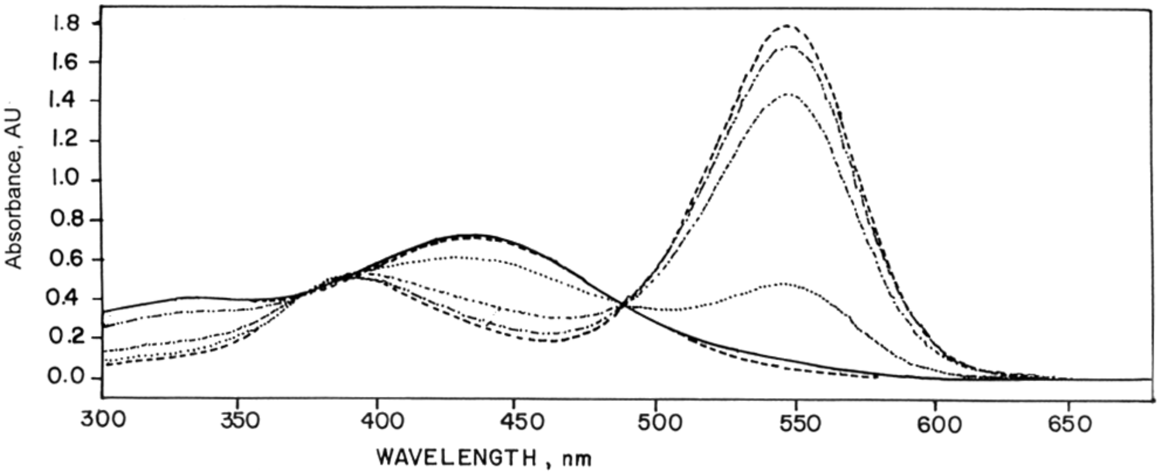
For each sample set prepared, the following are required. Each student or team of students will need to prepare one complete sample set.

- 10-mL volumetric flasks, (15)
- 100-mL volumetric flask, (1)
- glass capillary tubing or disposable glass pipets (3-4 for each student)
- buret, buret clamp and ring stand
- wash bottle, filled with distilled, de-ionized (DI), carbonate-free water
- laboratory tissue for wiping pipet tips and cuvettes
- poly bottles for solution storage

Our students have access to a spreadsheet application (Excel) and a mathematical application (MathCad); however, all calculations can be performed without these, in the time-honored "manual" method, or with a hand-held calculator.

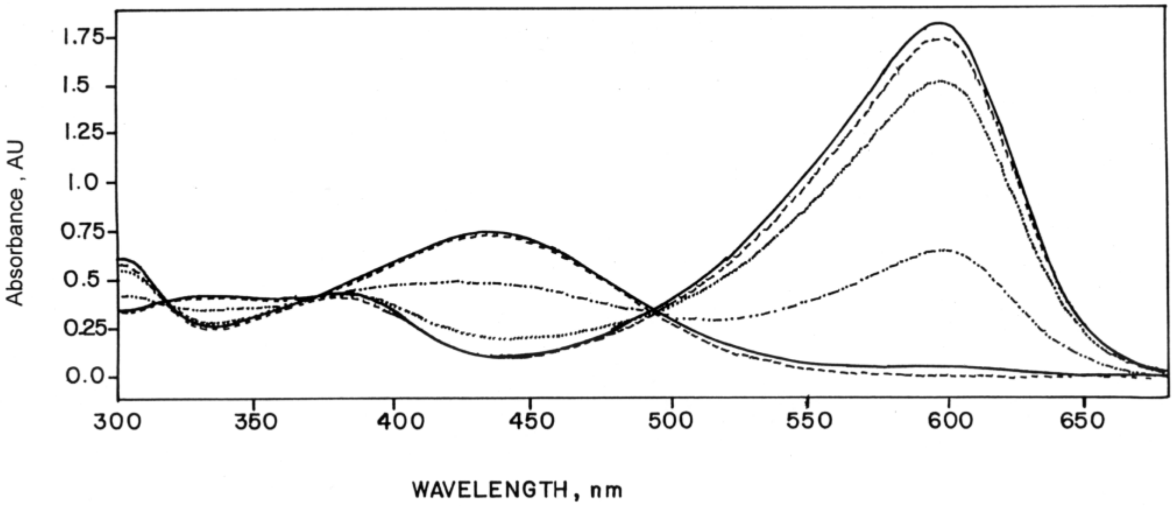
Generating the Titration Curve

Potentiometric titration of a dilute dye solution (~ 25 mg in 0.1 L DI water, precisely known) is performed using 0.02 M NaOH solution. A 0.1 M (precisely known) NaOH solution is prepared and standardized in the usual manner against primary standard KHP (potassium acid phthalate), and quantitatively diluted to 0.02 M. The dye solution is adjusted to pH ≤ 2 before starting the titration. A 30.00-mL aliquot of this dye solution will require about 30.00 mL of the 0.02 M NaOH to reach the second equivalence point. Visual observation of



pH	Abs., 432 nm	Abs., 545 nm	Abs., 595 nm
- - - - - 0.0	0.285	1.788	0.254
··–··–0.59	0.316	1.68	0.242
–·–·–0.96	0.399	1.43	0.200
·········1.92	0.607	0.477	0.053
———3.02	0.712	0.096	0.000
- - - - -3.92	0.699	0.058	0.004

Figure 2: Student-generated plot of overlay spectra, pH 0 to pH 4.



pH	Abs., 432 nm	Abs., 545 nm	Abs., 595 nm
———5.99	0.734	0.056	0.007
- - - - -6.98	0.715	0.041	0.000
·········8.00	0.722	0.072	0.004
–··–··–8.95	0.484	0.366	0.640
·········9.96	0.201	0.814	1.513
———11.38	0.109	0.969	1.813
- - - -12.80	0.100	0.924	1.732

Figure 3: Student-generated plot of overlay spectra, pH 6 to pH 13.

color, titrant volume, and pH are recorded during the titration. For best results, the volume of each titrant addition should be kept small (≤ 0.5 mL).

Generating the Beer's Law Calibration Curve

Three sets of dilutions are prepared, five dilutions per set, at each of three pHs, according to the example in Table 1. A detailed procedure provided to students is available in the supplementary material (Student Handout, 42dr1897.pdf).

Absorbance is measured for each solution at the maximum wavelengths for the yellow, red, and blue forms (436 nm, 545 nm, and 595 nm, respectively). Examples of student data for this portion of the experiment are available in the supplementary material (Excel files containing data sheets and charts, 42dr2897.zip).

Determining the α-Functions Through the Range pH 0 to 13. A second set of 14 solutions is prepared having equal concentrations of thymol blue and varying pH through the range from 0 to 13, one solution at each (approximately) integer pH value. The concentration of this set should not be greater than ~3×10^{-5} M, to keep the absorbance of the blue form low enough for good readings on the spectrophotometer. In order to examine the isosbestic points, the entire spectrum is recorded from 350 to 650 nm for each sample.

Data Reduction and Presentation

Absorbance data for the pure solutions at pH 0.0, 6.0, and 13.0 is recorded at 436, 545, and 595 nm. A spreadsheet application is used to plot the three families of calibration curves and determine molar absorptivities of each species at each wavelength. A summary of one student's data and the calibration curves generated from this data are provided in the supplementary material (42dr2897.zip).

Titration data is plotted as usual, pH versus volume of titrant added, and a spreadsheet application is used to manipulate the data. Students compute and plot the algebraic approximation of first and second derivatives and a Gran's plot. Depending on the care taken, some students may see a small break in the curve at about pH 5.6, the first equivalence point; however, most students do not see this break. Students enter their color observations on the plot and identify the species present in the various regions. An example of student data is available in the supplementary material (42dr2897.zip).

The spectra showing the color transitions are overlaid, and if quantitative laboratory protocols have been carefully followed the isosbestic points superimpose nicely. Students prepare two sets of overlay plots showing the transitions from red to yellow, and from yellow to blue (Figures 2 and 3).

Data at the absorbance maxima of the three species are analyzed for the fraction of each species present in each solution using MathCad to solve the matrix algebra. The fractions of each species are plotted against pH and the family of curves overlaid on the same axes (Figure 4). From the graph, students are asked to identify the two acid-dissociation equilibrium constants (K_a's), the pH of the first equivalence point (ampholyte species), and the pH regime in which each species is predominant. In the example provided, an art student with a background in color science identified the colors recorded for these solutions.

The concentration of each species present is computed from the Beer's law relationship using the spectroscopic data. The α-functions are then computed as the fraction of each species relative to the sum of all TB species present. For example, the fraction of the fully protonated species H_2TB, is given by

$$\alpha_0 = \frac{[\mathrm{H_2TB}]}{[\mathrm{H_2TB}]+[\mathrm{HTB^-}]+[\mathrm{TB^{2-}}]}$$

The calculation of these functions is presented in all standard quantitative analysis textbooks.

Practical Considerations

This experiment requires about 12 hours for a student to perform individually; however, by working in teams, dividing the tasks, and sharing the data, a class can perform the experiment in about six laboratory hours. If enough spectrophotometers were available, and each member of the class were responsible for only one or two solutions, including those measurements, the data could be assembled in about three to four hours. In our procedure, one team member prepares three sets of TB concentration standards and records absorbences at the three selected wavelengths for each sample. The pH 0.0 solutions (species H_2A) and pH 13.0 solutions (species A^{2-}) are quickly adjusted for pH; however, more time and patience are required for the midrange set at pH ~6.0, the ampholyte species, as these tend to be "jumpy."

A second student standardizes the base and performs the potentiometric titration. A third student prepares the 14 equal-concentration solutions spanning the pH range and records absorbance of each at the three wavelengths. A fourth student could be responsible for reduction of this data to determine the fraction of each species present at each pH. Our students share all data, but each is required to prepare an individual write-up. Alternatively, the students could assemble the data and report on it as a team.

Conclusions

This experiment goes beyond the traditional weak-acid titration, introduces common laboratory instrumentation, and illustrates how a more complete picture can be derived using complementary data from different experiments. Good quantitative laboratory protocols must be observed if satisfactory results are to be achieved. The instructor can tell at a glance if the student has performed the experiment with careful attention to analytical laboratory protocols: the spectral overlays reveal if all solutions were equal concentration, the calibration curves show if dilutions were properly made, and from the α-plots it can be readily seen if the quantitative solution transfers and pH adjustments were properly performed.

Thymol blue is especially suited to this experiment because its spectral response follows Beer's law through a wide range of concentrations and because the acid dissociation constants are separated by several orders of magnitude. Very elegant and precise work reported in the literature provides reliable values for the "answers" [16].

The students' response to this experiment has been favorable, and they indicate that it clarifies some difficult concepts. Our students state they have a greatly enhanced understanding of the information displayed in the fraction versus pH curves and how these curves present complementary data to the more familiar titration curve. Correlating the two plots with each other and including the color observations on the two plots helps students achieve an intuitive grasp of the equilibria involved and the predominate species at each pH. Because there are no more than two species present in appreciable concentrations at any one time during a titration of

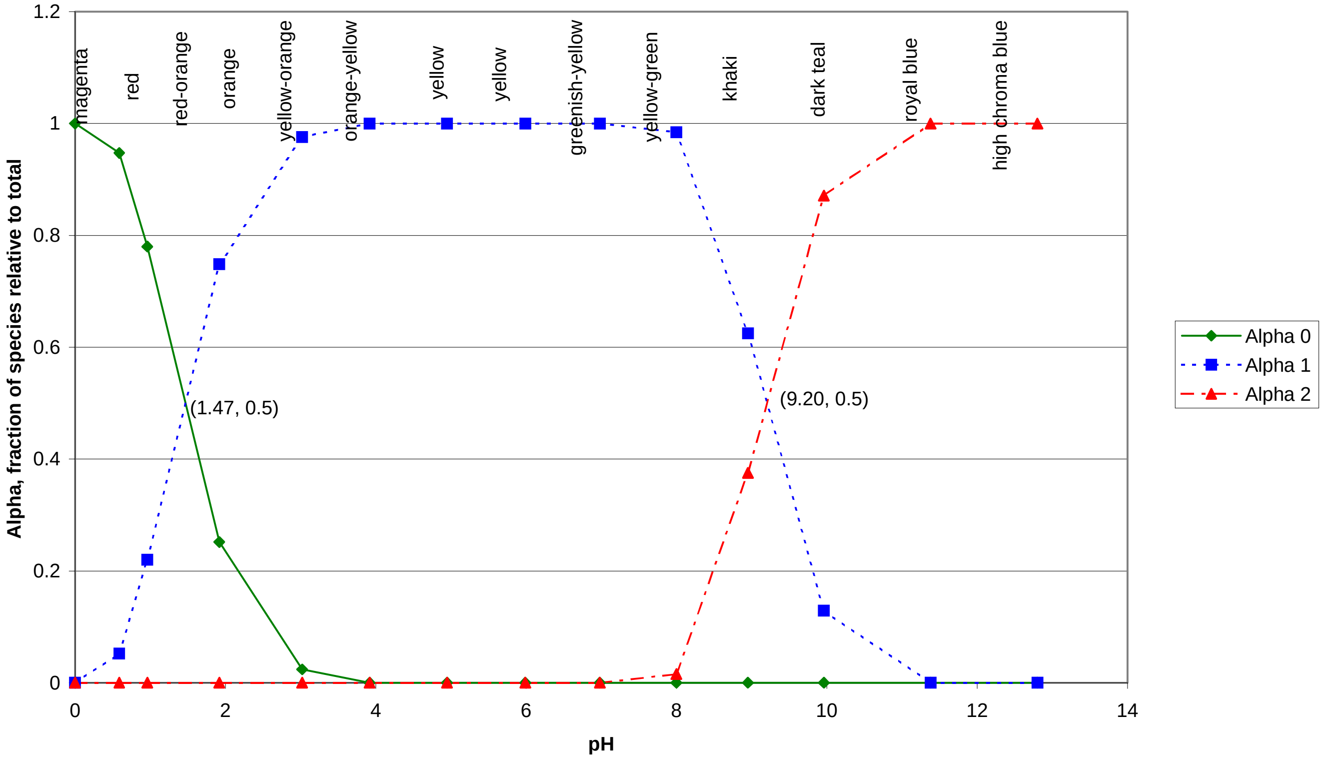


Figure 4. Alpha functions for thymol blue.

TB, students can understand the isosbestic points in the spectra. Students also seem to enjoy the vivid colors of the various forms of the dye, and they appreciate the fact that those colors provide a direct visual indication of the species involved.

This experiment addresses traditional goals of the quantitative analysis laboratory. At the same time, it reflects current efforts to modernize this course to include instrumentation, spreadsheets, mathematical application programs, and the concept of working in teams. It also emphasizes to students the importance of careful solution manipulations if instrumental data are to be meaningful. Finally, the application to environmental analysis increases the interest level for many students.

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