Spectrophotometric Determination of the Dissociation Constant of an Acid–Base Indicator Using a Mathematical Deconvolution Technique

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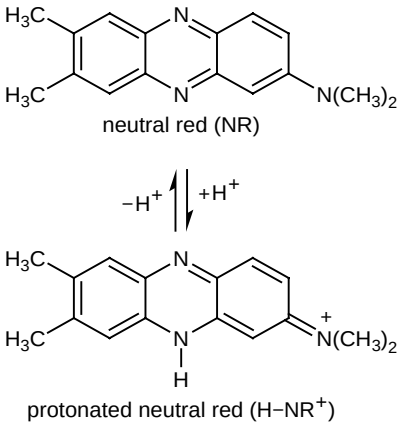
Determining the acid-dissociation constant (K_a) of a chemical species is one of the more common laboratory experiments in the undergraduate chemistry curriculum. This is undoubtedly due to the fact that an understanding of acid–base equilibria provides a foundation for exploring more complex chemical concepts. Acid-dissociation constants can be measured by a variety of methods including potentiometric *(1)* or conductimetric *(2)* titration, spectrophotometry *(3–7),* fluorescence spectroscopy *(8, 9),* or chromatography *(10).* In this experiment, we focus solely on the spectrophotometric determination of a K_a value as a means for students to explore a familiar concept from their introductory chemistry course while using more complex instrumentation and data analysis techniques within an upper-level course such as quantitative analysis, instrumental methods of analysis, or physical chemistry.

One of the most common laboratory experiments for the spectrophotometric determination of an acid-dissociation constant involves using an acid–base indicator species *(3–6).* Students initially collect absorbance spectra for the indicator in its purely acidic (HIn) and basic (In^-) forms. Based on these spectra, students choose two wavelengths for further analysis and prepare a series of indicator solutions of varying pH where the acidic and basic forms are simultaneously present. Students then measure the absorbance of their indicator solutions at both of their chosen wavelengths and, from this, determine the concentration of the acidic and basic forms of the indicator present at each pH. Finally, they calculate the acid-dissociation constant based on a plot of log $[In^-]/[HIn]$ as a function of the pH of the solution *(4, 5).*



Scheme I. The protonation and deprotonation reaction of neutral red.

While these methods are straightforward experimentally, they are often difficult for students to conceptually understand. As a result, we have developed an experiment to determine the K_a of an acid–base indicator species using a similar spectrophotometric method but incorporating a unique data analysis technique. A mathematical deconvolution program is used so that students may resolve individual spectral components within complex absorbance spectra. If two species in a solution have overlapping individual absorbance spectra, the measured absorbance of the mixture will be a convolution of the individual spectra. Because the convolved spectra can be described by a mathematical function, deconvolution is essentially a mathematical "undoing" of the two spectra into individual components.

In this experiment, when the acidic and basic forms of the indicator are simultaneously contributing to the overall absorbance spectrum, deconvolution allows students to mathematically separate the two spectral components and quantify their contribution. This data analysis technique has an inherent advantage over other methods used to determine the K_a of an acid–base indicator species because it allows students to *visually observe* how the pH of a solution affects the absorbance of the acidic and basic forms of the indicator.

Background

To spectrophotometrically determine the acid-dissociation constant for a species, ideally, it should have spectrally distinct acidic and basic forms *(11).* For example, the indicator that we have chosen for this experiment, neutral red,[1] has an acidic absorption maximum at 525 nm and a basic absorption maximum at 450 nm. The reaction for the protonation and deprotonation of neutral red is shown in Scheme I. The equilibrium constant for this reaction, known as the acid-dissociation constant (K_a), is defined as,

$$K_a = \frac{a_{NR}\, a_{H^+}}{a_{H\text{–}NR^+}} \qquad (1)$$

where a_x represents the activity of each individual species, x, in the protonation reaction. This expression is often simplified by incorporating molar concentrations (commonly denoted with brackets) in the place of activity coefficients for each species. This is the apparent acid-dissociation constant (K_a'):

$$K_a' = \frac{[NR][H^+]}{[H\text{–}NR^+]} \qquad (2)$$

Taking the logarithm of eq 2 and rearranging the expression gives the equation:

$$pH = pK_a' + \log \frac{[NR]}{[H\text{–}NR^+]} \quad (3)$$

Closer examination of the equation shows that when the concentrations of H–NR$^+$ and NR are identical, the pH of the solution is equal to the pK_a' value for the indicator.

We have used this relationship as the basis for this experiment. Students first measure the absorbance of the neutral red indicator in its purely acidic and basic forms and determine the molar absorptivities of each species by preparing Beer's law plots. Students then measure the neutral red absorbance in solutions of varying pH in which NR and H–NR$^+$ are simultaneously present. Finally, students use a commercial software program, PeakFit,[2] to deconvolve each neutral red absorbance spectrum into its distinct components. They use the resolved spectra to assess the quantity of NR and H–NR$^+$ present at each pH studied, correcting for differences in the molar absorptivities of each species. By constructing a plot of the percentage of NR and H–NR$^+$ as a function of solution pH, the pK_a' is determined as the pH where the quantities of NR and H–NR$^+$ are equal.

Experimental Procedure

Buffers should be prepared for the students at a variety of pHs but at constant concentration (we used 0.2 M) prior to the laboratory period. For our experiments, citric acid buffers (pH range = 3 to 5), phosphate buffers (pH range = 6 to 8), and boric acid–borate buffers (pH range = 8 to 10) were used.

The neutral red indicator is prepared prior to the laboratory period as a stock solution in absolute ethanol. The students then use the stock to prepare neutral red solutions in a range of pH buffers by first pipetting the appropriate aliquot of stock solution directly into a cuvette, evaporating off the ethanol with a gentle stream of nitrogen gas, and then pipetting the appropriate quantity of buffer into the cuvette. Disposable methacrylate cuvettes with a 1-cm pathlength are used for the preparation of all solutions.

Students are first asked to choose the appropriate pH buffers to generate absorbance spectra of the neutral red in its purely acidic and basic forms. After doing so, they are directed to determine the molar absorptivities of NR and H–NR$^+$ by preparing Beer's law plots for both species (a prelaboratory assignment submitted on the day of the experiment provides a review of the Beer–Lambert law and its applications). Students are then asked to prepare five solutions at pH values in which NR and H–NR$^+$ are present simultaneously and measure the absorbance of each.

All neutral red absorbance data are directly imported into the mathematical deconvolution program.[2] Students then have the opportunity to smooth their spectra and to fit their data using several different models (e.g., Gaussian, Lorentzian, etc.). Students determine the "goodness of fit" for each of their analyses by achieving a maximum R^2 value (where R^2 = 1 for a perfect fit and R^2 = 0 for a complete lack of fit), retaining random fit residuals around zero, and using the fewest number of spectral components. The PeakFit program that we use then allows students to determine the integrated peak area and peak maximum for each deconvolved component.

Because absorbance (A) increases linearly with the concentration (c) of species present according to the Beer–Lambert law ($A = \varepsilon bc$, where ε is the molar absorptivity and b is the cell pathlength), a larger integrated peak area is indicative of an increased concentration of species (assuming ε and b are constant). However, because the molar absorptivities of H–NR$^+$ and NR differ by a factor of almost two, it is necessary for students to account for the ε values when comparing the peak areas of the two species (we have students calculate a molar absorptivity "scalar" that is then multiplied by all of the peak area data for either H–NR$^+$ or NR). Students calculate the percentage of NR and H–NR$^+$ by dividing the molar absorptivity-corrected peak area for each species by the total peak area for each solution analyzed. Students then prepare a plot of the percentage of NR and H–NR$^+$ as a function of pH in order to determine the pK_a'.

Hazards

Hazards associated with this experiment are minimal. However, caution should be exercised when handling strongly acidic or basic solutions. Also, neutral red may cause skin and eye irritations, so typical safety precautions should be used.

Results

The absorbance of neutral red in acidic (pH = 3) and basic (pH = 9) media along with typical absorption maxima (λ_{max}) for each species, λ_{max} (NR) = 450 nm and λ_{max} (H–NR$^+$) = 525 nm, are shown in Figure 1. After determining a pH where neutral red exists in its purely acidic and basic forms,

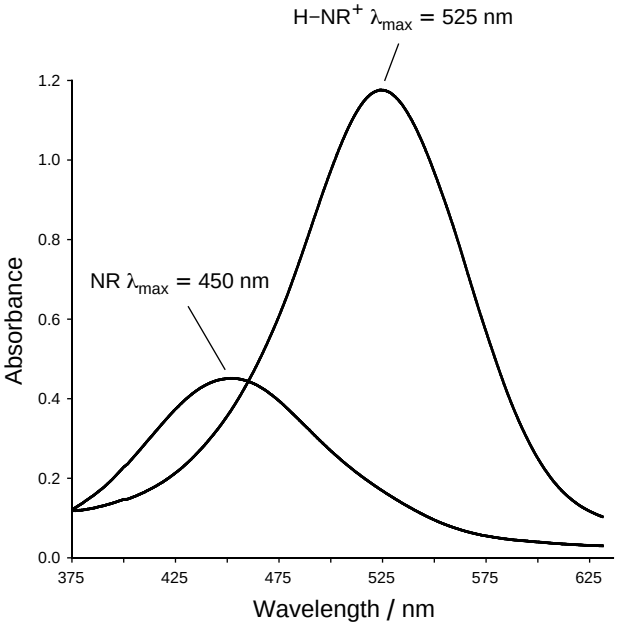


Figure 1. Neutral red absorbance in acidic (pH = 3) and basic (pH = 9) media. The absorbance maxima (λ_{max}) for the acidic (H–NR$^+$) and basic (NR) forms of the indicator are denoted.

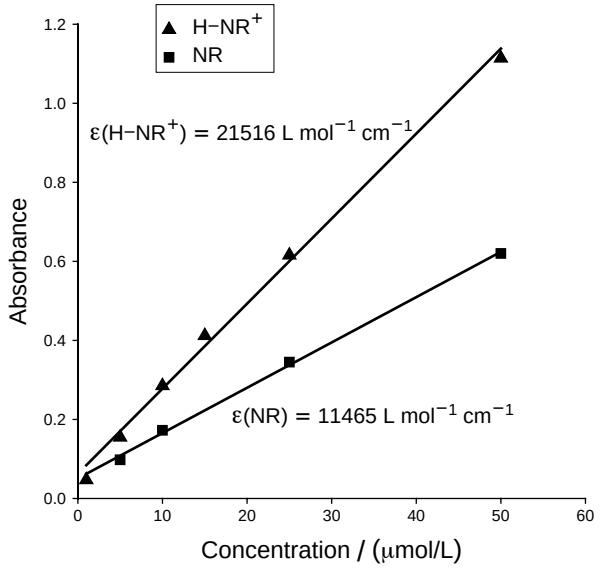


Figure 2. Beer's law plot for neutral red in acidic (pH = 3) and basic (pH = 9) media. Based on this plot, the molar absorptivities (ε) were determined for the protonated (H–NR+) and deprotonated (NR) forms of the indicator.

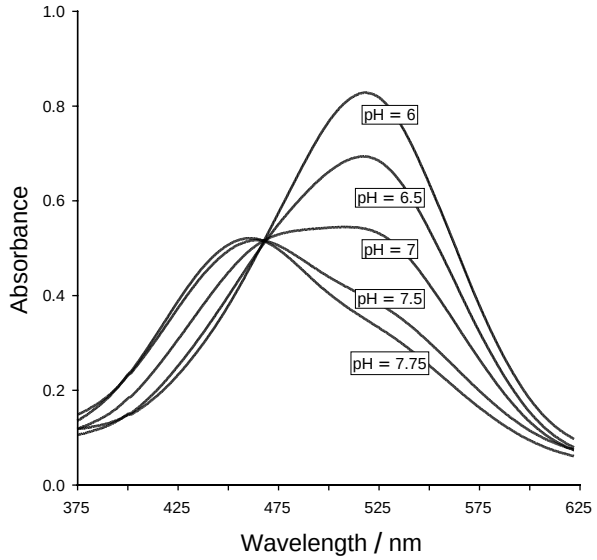


Figure 3. Student-generated absorbance data for neutral red in various pH buffers. The isosbestic point at 466 nm is indicative of the presence of two chemical species (the acidic and basic forms of the indicator) in this system.

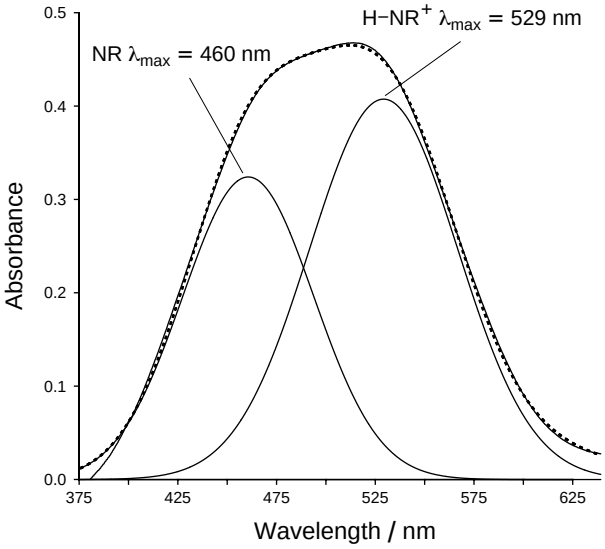


Figure 4. A typical student's spectral deconvolution of the neutral red absorbance (pH = 7.0). The resolved absorbance maxima (λ_{max}) for the acidic (H–NR+) and basic (NR) forms of the indicator are denoted. The dashed line indicates the theoretical fit to the data. The R^2 value for the fit is 0.998.

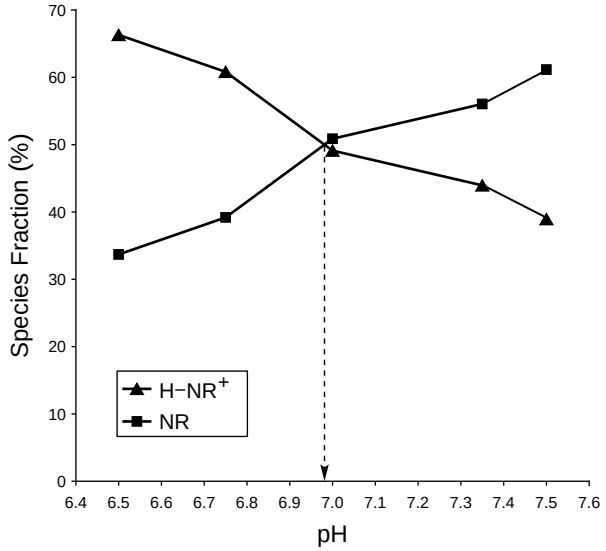


Figure 5. Representative student data showing the percentage of neutral red in its acidic (H–NR+) and basic (NR) forms as a function of solution pH. The pH where the percentages of the acidic and basic forms are equal apparent pK_a (pK_a′ = 6.98) of the NR indicator.

students prepare Beer's law plots (Figure 2) to determine the molar absorptivities for NR and H–NR$^+$: for Figure 2, ε_{NR} = 11465 L mol^{-1} cm^{-1} and ε_{H-NR^+} = 21516 L mol^{-1} cm^{-1}.

The neutral red absorbance data in solutions of varying pH are shown in Figure 3. The isosbestic point at 466 nm is indicative of the presence of two chemical species—in this case, the acidic and basic forms of the indicator. Such data are then imported into the spectral deconvolution program and analyzed. As shown in Figure 4, students deconvolve two absorbance peaks with maxima that correspond to those for NR, λ_{max} (NR deconvolved) = 460 nm, and H–NR$^+$, λ_{max} (H–NR$^+$ (deconvolved) = 529 nm.

After determining the best model to fit their data, students integrate the area under each deconvolved peak, correct their data using the molar absorptivities determined from their Beer's law plots, and calculate the percentage of NR and H–NR$^+$ at each pH studied. These data are then plotted (Figure 5), and the pK_a' value for neutral red is determined. For example, the student data in Figure 5 give a neutral red pK_a' value of 6.98, a value well within the range of the literature (6.5 to 7.4) *(12–14).* In their laboratory report, students are asked to discuss sources of error in the experiment and the data analysis technique that contribute to deviation of their results from published values.

Conclusion

This laboratory experiment reinforces the concept of acid–base equilibria while introducing a common application of spectrophotometry and can easily be completed within a standard four-hour laboratory period. It provides students with an opportunity to use advanced data analysis techniques such as data smoothing and spectral deconvolution to conceptualize the equilibrium that exists between the acidic and basic forms of an indicator. Although the instrumental measurements are similar to those previously reported *(4, 5),* the mathematical deconvolution technique significantly improves these methods by allowing students to directly observe and quantify how the absorbances of the acidic and basic forms of the indicator are individually affected by the pH of a solution.

Acknowledgments

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[w]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

Notes

1. Although we use neutral red, a number of other acid–base indicators would be appropriate for this experiment as well.
2. We use PeakFit (SYSTAT Software, Inc.; Richmond, CA) but Microsoft Excel Solver has similar capabilities.

Literature Cited

1. Potentiometric methods are extremely common, especially in the general chemistry and quantitative analysis laboratory. For example, see: (a) Cawley, J. J. *J. Chem. Educ*. **1993,** *70,* 596–598. (b) Harris, D. C. *Quantitative Chemical Analysis,* 6th ed.; W. H. Freeman and Company: New York, 2003; p 852. (c) Day, R. A.; Underwood, A. L. *Quantitative Analysis Laboratory Manual,* 6th ed.; Prentice Hall: Englewood Cliffs, NJ, 1991; pp 142–144.
2. Kooser, A. S.; Jenkins, J. L.; Welch, L. E. *J. Chem. Educ*. **2001,** *78,* 1504–1506.
3. Tobey, S. W. *J. Chem. Educ*. **1958,** *10,* 514–515.
4. Sawyer, D. T.; Heineman, W. R.; Beebe, J. M. *Chemistry Experiments for Instrumental Methods,* 1st ed.; John Wiley & Sons: New York, 1984; pp 193–198.
5. Patterson, G. S. *J. Chem. Educ.* **1999,** *76,* 395–398.
6. Williams, K. R.; Tennant, L. H. *J. Chem. Educ*. **2001,** *78,* 349–351.
7. Cavaleiro, A. M. V.; Pedrosa de Jesus, J. D. *J. Chem. Educ*. **2000,** *77,* 1218–1220.
8. Ribou, A.-C.; Vigo, J.; Salmon, J.-M. *J. Chem. Educ*. **2002,** *79,* 1471–1474.
9. Gsponer, H. E.; Argüello, G. A.; Argüello, G. A. *J. Chem. Educ*. **1997,** *74,* 968–972.
10. Hein, J.; Jeannot, M. *J. Chem. Educ*. **2001,** *78,* 224–225.
11. A number of techniques have been developed to determine K_a values for species with overlapping acidic and basic forms. For example, see: (a) Thamer, B. J.; Voigt, A. F. *J. Phys. Chem.* **1952,** *56,* 225–232. (b) Kara, D.; Alkan, M. *Spectrochim. Acta A* **2000,** *56,* 2753–2761. (c) Cladera, A.; Tomas, C.; Estela, J. M.; Cerda, V. *Anal. Chim. Acta* **1994,** *286,* 253–263.
12. Drummond, C. J.; Greiser, F.; Healy, T. W. *J. Chem. Soc. Faraday Trans*. **1989,** *85,* 551–560.
13. *The Merck Index,* 12th ed.; Merck & Co., Inc.: Rahway, NJ, 1983; p 6325.
14. Dutta, R. K.; Bhat, S. N. *Can. J. Chem.* **1993,** *71,* 1785–1791.