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Fitting Imidazole ^{1}H NMR Titration Data to the Henderson–Hasselbalch Equation

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ABSTRACT: A laboratory project in which students fit ^{1}H NMR data from the pH titration of imidazole to a polynomial equation has been described in this *Journal* by W. J. Hagan, Jr. et al. In this communication, fitting the experimental data to a theoretically derived equation, based on the Henderson–Hasselbalch equation, is examined and arguments in favor of this type of fit are presented.

KEYWORDS: *First-Year Undergraduate/General, Second-Year Undergraduate, Upper-Division Undergraduate, Analytical Chemistry, Biochemistry, Laboratory Instruction, Organic Chemistry, Acids/Bases, NMR Spectroscopy, Titration/Volumetric Analysis*

Hagan et al. published in this *Journal* a wonderful, straightforward laboratory project using ^{1}H NMR to follow the pH titration of imidazole.[1] A few suggestions to improve the data analysis aspect of this experiment are presented. Hagan et al. displayed a "calibration" curve showing the influence of pH on the chemical shift of the imidazole proton on the C2 carbon (between both ring nitrogens) in their Figure 2 and reproduced in Figure 1 here. Their curve

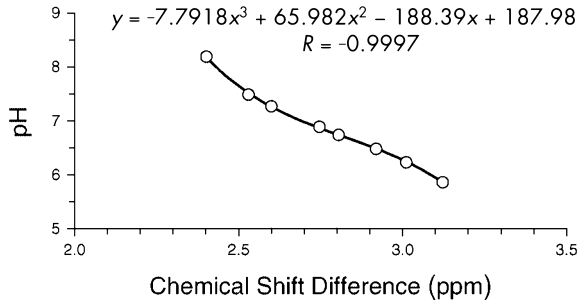

Figure 1. The calibration curve from Hagan et al. (Reprinted from ref [1]. Copyright 2007 Division of Chemical Education, Inc.). The chemical shift difference is between the proton on the imidazole C2 and the water peak.

plotted pH on the *y* axis versus chemical shift difference (ppm) on the *x* axis, and the data were fit to a third-order polynomial. Students then used this calibration curve to determine the pH of unknown imidazole solutions.

Difficulties both with the plot and the polynomial fit argue against having students analyze their data in this fashion. Because the experimenter makes the buffers and directly controls the pH, this is the independent variable and should, therefore, be plotted on the *x* axis; chemical shift, which depends on pH, is the dependent variable and should be plotted on the *y* axis.[2] An even more important problem with this "calibration" curve is the third-order polynomial fit. Although the polynomial equation fit the data well,[3] it seems unwise to encourage students to use theoretically meaningless fit equations, especially when a robust, theoretically defensible equation is available. For pH titrations, the Henderson–Hasselbalch equation should be used to predict protonation-influenced changes with pH. In fact, Gasparovic et al.,[4] who were cited by Hagan et al. as an original source for their project, fit their results using eq 1

$$\mathrm{pH} = \mathrm{p}K_a + \log[(y_{max} - y)/(y - y_{min})] \quad (1)$$

where *y* is the experimentally measured parameter (chemical shift, in this case), and $(y_{max} - y)$ and $(y - y_{min})$ are proportional to the concentrations of the deprotonated and protonated forms of the acid, respectively.[5,6] The simulated titration in Figure 2 helps to explain these quantitative relationships.

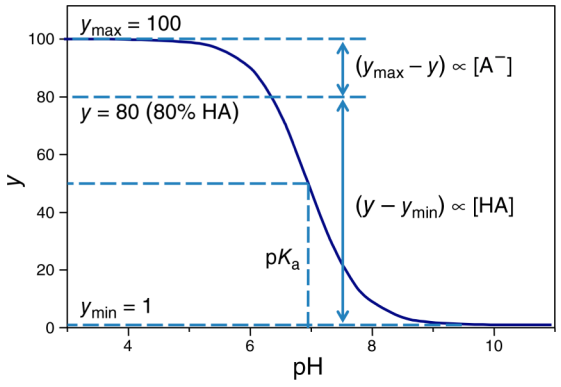

Figure 2. Simulated titration of an acid (HA) with pK_a = 7.0, y_{max} = 100, and y_{min} = 1. Note that for the example of y = 80, i.e., 80% HA and 20% A^-, it can be seen that $[A^-] \propto (y_{max} - y)$ and $[HA] \propto (y - y_{min})$.

To fit the data set for pH versus measured *y* value, the Henderson–Hasselbalch equation must be recast in the form $y = f(x)$ (eq 2):[7]

$$y = y_{min} + (y_{max} - y_{min})/(1 + 10^{(\mathrm{pH} - \mathrm{p}K_a)}) \quad (2)$$

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A fit of eq 2 to the data set of Hagan et al. (Figure 1) is shown in Figure 3 .

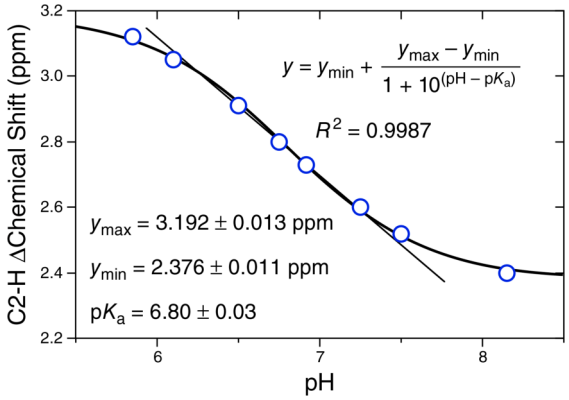

Figure 3. Calibration curve data from Hagan et al. fitted to eq 2. This plot and fit were generated using Kaleidagraph.[8].

There are several advantages to using the Henderson–Hasselbalch equation to fit the titration results. Most importantly, students are encouraged to fit their data to an equation derived for the actual chemical system that they are studying. Furthermore, once they have best-fit values for y_{min}, y_{max}, and pK_a, they can easily use the Henderson–Hasselbalch equation (eq 1) to calculate solution pH from the experimentally determined imidazole C2 proton chemical shift. As an added bonus, students can compare the best-fit value of the pK_a for their data set with literature values. For example, from the fit in Figure 3, pK_a(imidazole) = 6.80 ± 0.03 can be compared to the value of 6.95 (at T = 25 °C) quoted by Hagan et al., and the value of 6.99 found in the literature.[9]

A final question one must ask at this point is the pedagogical one: Which students should be asked to fit their data to an equation such as Henderson–Hasselbalch, or for that matter, a third-order polynomial? One of the unique aspects of the laboratory project described by Hagan et al. was its suitability not only for upper-division students, but also for first- or second-year students. At this lower level, it seems unwise to ask students to apply an advanced fitting program to their data to determine best-fit parameters that they can then use. First- and second-year students should be encouraged to use pairwise linear interpolation between nearest points or what Hagan et al.[1] refer to as "manual calibration". This encourages students to use their calibration curve in the simplest way possible, without bothering with the added complexity of a fit equation. As Hagan et al.[1] showed in their Tables 1 and 2, the difference in prediction error when using a third-order polynomial fit versus the "manual calibration" method was statistically insignificant. Alternatively, students can be encouraged to use linear regression for titration results that lie within the linear range (pH 6.2–7.4 in Figure 3) and pairwise linear interpolation for results outside of this linear range. Perhaps the simplest alternative is to have students print out the chemical shift versus pH "calibration" plot with gridlines and use a flexi-curve (bendy-spline) to draw a fitted curve. For any sample chemical shift, the pH can then be read from the x axis. It goes without saying that upper-division students who carry out this excellent project should be encouraged to apply eq 2 and nonlinear curve-fitting to their data.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Hagan, W. J., Jr.; Edie, D. L.; Cooley, L. B. *J. Chem. Educ.* **2007**, *84*, 1188–89.

(2) One could argue that, because regression algorithms assume all error to be in the experimental y values, one should put the parameter with the smallest experimental uncertainty on the x axis. For Hagan et al.'s imidazole NMR titation, chemical shifts of 3.000 ± 0.001 ppm have a smaller uncertainty than pH's of 7.00 ± 0.01. On the other hand, spectrophotometric calibration curves always plot the dependent variable, absorbance, on the y axis, even though absorbance uncertainties (e.g., 1.000 ± 0.002) can sometimes be smaller than concentration uncertainties (e.g., 1.000 ± 0.010 M).

(3) An R value of −0.9997 was reported, which gives an R^2 of 0.9994.

(4) Gasparovic, C.; Barba, I.; Born, J.; Barton, S.; Arus, C.; Mann, P. *Anal. Biochem.* **1998**, *261*, 64–72.

(5) Note that this equation applies to the situation where y decreases as pH increases, as is the case for the imidazole chemical shift. For the opposite case, where y increases with pH, there would be a minus sign in front of the log term, instead of a plus sign.

(6) Silverstein, T. P.; Blomberg, L. E. *J. Chem. Educ.* **1992**, *69*, 852–855.

(7) Once again, this equation applies to the situation where y decreases as pH increases, as is the case for the imidazole chemical shift. For the opposite case, where y increases with pH, the exponent would be pK_a − pH, instead of pH − pK_a. Equation 2 can alternatively be written as $y = (y_{max} + y_{min}10^{(pH-pK_a)})/(1 + 10^{(pH-pK_a)})$.

(8) Many graphing programs include similar nonlinear curve-fitting algorithms and could be used to fit the data: e.g., Excel's SOlver, SigmaPlot, PeakFit.

(9) Fasman, G. D., Ed. *CRC Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Vol. *1*, Physical and Chemical Data; CRC Press: Boca Raton, FL, 1976; p 212.