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Experimental Determination of pKa Values by Use of NMR Chemical Shifts, Revisited

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Abstract

This laboratory experiment, using proton NMR spectroscopy to determine the dissociation constant for heterocyclic bases, has been modified from a previously described experiment. A solution of a substituted pyridine is prepared using deuterium oxide (D_2O) as the solvent. The pH of the solution is adjusted and proton NMR spectra are collected for a variety of pH values. The chemical shifts of the peaks in the NMR spectrum change depending on the degree of protonation of the pyridine ring. Analysis of the spectral data is used to calculate the dissociation constant of the substituted pyridine. This experiment is suitable for a variety of advanced chemistry laboratories including analytical chemistry and instrumental analysis.

Graphical Abstract

Keywords

Upper-Division Undergraduate; Analytical Chemistry; Laboratory Instruction; Acids/Bases; Aromatic Compounds; Equilibrium; Instrumental Methods; NMR Spectroscopy; pH; Quantitative Analysis.

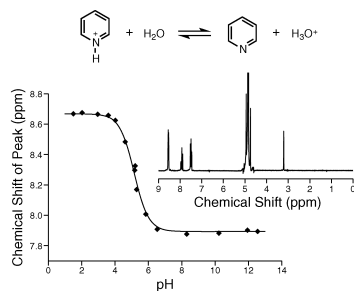# Experimental Determination of pK_a Values by Use of NMR Chemical Shifts, Revisited

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Nuclear magnetic resonance (NMR) is a powerful and common tool used in a variety of disciplines; however, many chemistry majors may have limited experience operating an NMR during their undergraduate career. Students generally get exposure to NMR spectra in introductory-level organic classes and laboratories but student access to the NMR spectrometer is usually limited. For example, at our university, organic students submit samples to their lab instructor for analysis and receive the spectrum of their submitted sample at a later time for interpretation. Although this procedure is necessary due to time constraints and logistical reasons, it does not allow students hands-on opportunities to collect data with the NMR spectrometer. Another opportunity for chemistry majors to gain experience using an NMR spectrometer is in the instrumental analysis laboratory; however, NMR is often not part of the instrumental analysis curriculum. This was true at our university until a few years ago so graduating chemistry majors had limited to no experience operating an NMR spectrometer. When searching for an NMR experiment to add to our instrumental analysis course, there were only a limited number of published analytical NMR experiments from which to select. A few examples in the past 20 years have used ^{1}H NMR spectra for the quantification of components in a mixture (*1-3*) and other experiments have used ^{13}C and ^{27}Al NMR spectra as a means for quantification (*4,5*).

The three-hour laboratory described here is a modification to a previously published laboratory. The original laboratory (*6*) was published in 1973 and was included in an instrumental analysis laboratory manual (*7*). However, the experiment used a 60 MHz scanning NMR and cannot be performed on an FT-NMR using a deuterium frequency-lock and deuterium shimming. This experiment describes modifications to Handloser's report (*6*) that include the use of deuterium oxide (D$_2$O) in the preparation of the samples and a changed procedure written to minimize the volume of D$_2$O used in preparing samples. Additionally, the laboratory is expanded to have students model the chemical shift of a proton at any pH and a correction is made to one equation in the original publication.

Background

The equilibrium between a heterocyclic base (B) and its conjugate acid (BH$^+$), such as pyridine (eq 1), will shift depending on the pH of the solution. As the pH of the solution is changed, the position of the proton peaks in the NMR spectrum will also shift. The chemical shift (ν) of the protons can be expressed by eq 2 where ν_{BH^+} and ν_B are the chemical shifts of the protonated and unprotonated forms, respectively, and x_{BH^+} and x_B are the mole fractions of the protonated and unprotonated forms, respectively.

$$\text{BH}^+ + H_2O \rightleftharpoons B + H_3O^+ \tag{1}$$

$$\nu = \nu_{BH^+} x_{BH^+} + \nu_B x_B \tag{2}$$

Using this information and the acid dissociation constant expression, the pK_a for the pyridinium ion can be determined using eq 3.

$$pK_a = pH + \log \frac{x_{BH^+}}{x_B} \tag{3}$$

70 (See the Supporting Information and previous publications (*6,7*) for more background details.)

The Experiment

A student, or group of students, is instructed to determine the pK_a for the conjugate acid of a methyl-substituted pyridine compound. In this article, pyridine is used as the example compound, but a list of methyl-substituted pyridine compounds that also work well for this experiment is shown
75 in Table 1. The solution is prepared by adding pyridine, D_2O, and methylammonium iodide to a test tube that is large enough to fit a pH probe. Tetramethylammonium iodide is added as a chemical shift standard instead of tetramethylsilane (TMS).

Table 1. List of Suitable Compounds Used with This Experiment with the Literature and Student pK_a Values

Compound	Literature p$K_a{}^a$	Student p$K_a{}^b$
Pyridine	5.18	5.16 ± 0.11
2-Picoline	5.96	5.98 ± 0.13
3-Picoline^c	5.66	—
4-Picoline^c	6.01	—
2,3-Lutidine	6.59	6.59 ± 0.05
2,4-Lutidine^c	6.68	—
2,5-Lutidine^c	6.44	—
2,6-Lutidine	6.69	6.73 ± 0.21
3,4-Lutidine^c	6.49	—
3,5-Lutidine	6.15	6.15 ± 0.05

80 ^{a} The average pK_a was used if more than one literature (*8–11*) value was available.

^{b} The student value is the mean pK_a determined for each set of equivalent ring protons. The uncertainty is the standard deviation of the pK_a values calculated from the pH range where the chemical shift is rapidly changing.

^{c} Student data for these compounds have not been collected with this modified lab, but are expected to work well due to structure similarity and results presented in original publication (*6*).

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The prepared pyridine solution has the pH adjusted multiple times. This is accomplished by preparing two hydrochloric acid solutions and two potassium hydroxide solutions with concentrations of approximately 1.0 M and 0.10 M. To minimize the water peak in the NMR spectrum, these acid and base solutions are prepared with D_2O. (See the Supporting Information for more details on acid and
90 base solution preparation.)

NMR sample spectra are collected for a wide range of pH values. The pH probe is inserted into the test tube with the sample and the pH is recorded. Next, a portion of the sample is placed in a NMR tube and a spectrum is collected for that specific pH value. The sample in the NMR tube is then returned to the test tube containing the sample and the sample pH is adjusted with either HCl or
95 KOH. When the solution has reached the desired pH, a portion of the sample is again placed in a NMR tube and a spectrum collected. This procedure is repeated until the spectra are collected for the desired number of pH values.

Hazards

The experiment should be carried out using proper protective eyewear. Concentrated hydrochloric
100 acid is corrosive and dilutions should be prepared in a fume hood. Potassium hydroxide is caustic. Pyridine and substituted pyridines are flammable, irritants, and can be absorbed through the skin. Students should wear protective gloves to prevent skin exposure. Students are instructed to collect used solutions in properly labeled containers so they can be collected by environmental health and safety at your institution.

Arrietta Clauss 7/25/12 4:48 PM
Comment [3]: Are the data in the table from one student or from the class average? This information should added to the text or as a note to the table.

Arrietta Clauss 7/25/12 4:57 PM
Comment [4]: This procedure appears to require each student or group of students to use the NMR multiple times during the lab; From the data in Figure 2, each student or group of students used the NMR at least 15 times during the lab. Can all the students complete this lab in one lab period or do the students sign up for NMR time? Please add some clarification to the text or in a note. How many students were in this lab?

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Comment [5]: The Hazard section was moved to follow the experimental section.

105   **Data Analysis**
      Analysis of the data requires determining the chemical shift of the pyridine ring protons for each
collected spectrum. A spectrum of pyridine (Figure 1A) shows how the large intensity of the water
peak at ~4.85 ppm dominates the spectrum. If the scale of the spectrum is expanded close to the
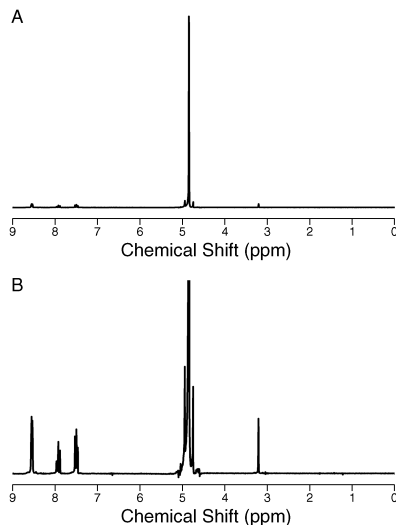baseline, the other proton peaks become more apparent (Figure 1B).

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Figure 1. ^{1}H NMR spectra of pyridine at pH = 6.53: (A) full-scale spectrum showing large water peak at 4.85 ppm and (B) expanded scale
spectrum showing tetramethylammonium reference protons at 3.207 ppm and the aromatic ring protons between 7 and 9 ppm.

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      After chemical shifts for all the spectra have been obtained, the students construct a graph of the
chemical shift of the ring protons as a function of pH. An example graph (Figure 2) examining the
peak at ~7.8 in Figure 1 shows little change in the chemical shift at high and low pH, but there is a
region of about 2 pH units where the chemical shift is rapidly changing (when the pH = $pK_a \pm 1$). The
120   values of the chemical shift at high pH (chemical shift in base) and low pH (chemical shift in acid) are
taken from the flat portions of the graph in Figure 2. For each data point on the curve, the x_B, x_{BH+},
and pK_a can be calculated using eqs 4, 5, and 3, respectively. Note that eq 4 is a correction to the
corresponding equation in the original publications (*6,7*).

125   $$x_B = \frac{\text{chemical shift in acid - observed chemical shift}}{\text{chemical shift in acid -chemical shift in base}} \qquad (4)$$

$$x_{BH^+} + x_B = 1 \qquad (5)$$

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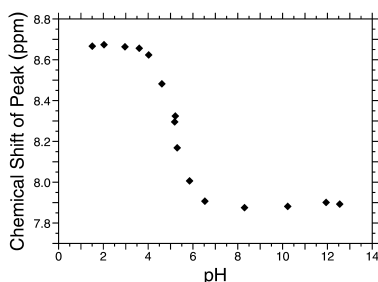**Comment [6]:** This phrase was added. Is it
accurate?

Figure 2. An example graph of the 1H NMR peak position of a ring proton of pyridine as a function of pH.

135   After the students have processed the data and obtained pK_a values for each data point in the rapidly changing portion of the graph, an average value can be calculated and the standard deviation can be used as an indication of uncertainty. The same process should be followed for each set of equivalent ring protons. Because these protons should all give the same value, the average pK_a for each set of equivalent protons will give the best estimate of the pK_a of the compound. Typical student
140   results are listed in Table 1 and demonstrate good agreement with the literature values.
       The students also model the chemical shift by using the equations given above. The students construct a spreadsheet with values for the chemical shift in acid, the chemical shift in base, and the pK_a obtained from their data analysis. Then, columns in the spreadsheet are prepared for pH, x_{BH+}/x_B, x_B, and ν. The pH column is incremented by 0.1 for the desired pH range and the columns for x_{BH+}/x_B,
145   x_B, and ν can be calculated by using eqs 3–5. Finally, the students are asked to plot the model data with their experimental data to see how the two compare. (See the Supporting Information for more details.)

ASSOCIATED CONTENT

Supporting Information. The laboratory procedure given to the students and instructor notes including answers
150   to the lab questions. This material is available via the Internet at http://pubs.acs.org.

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Comment [7]: This sentence was added.