NMReady

Determination of Acid Dissociation Constants via NMR Spectroscopy

Incorporating instrumentation into undergraduate laboratories increases students' understanding of fundamental and practical characterization techniques.

The NMReady™ benchtop spectrometer offers a portable and affordable option with a modern, network accessible, easy-to-use interface that can be easily incorporated into teaching laboratories.

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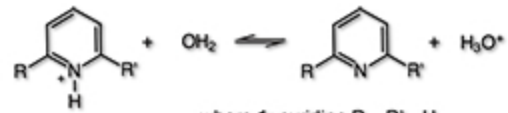
Introduction:

Given time restraints & limited access to equipment, it can be difficult to incorporate multidisciplinary undergraduate experiments into a laboratory curriculum. However, this type of experiment is useful to reinforce chemical concepts & help students discover connections that aid more advanced problem solving.

The following experiment combines analytical sample preparation, titrations & NMR spectroscopy to teach equilibrium, acid-base chemistry, biochemistry techniques & chemical shift dependence on pH. The ultimate goal is to determine the pK_a's for a series of nitrogen bases. It is suitable for a general, organic, analytical or physical chemistry lab.

NMR is typically discussed in a static sense; chemical shifts, integrations & multiplicities are typically reported on fixed structures. However, there are external factors (*e.g.*, concentration, temperature, pH) that can induce physical or chemical changes within a sample. Nitrogen bases, for example, can undergo pH-dependent chemical changes due to protonation of a lone pair on the nitrogen. At high pH the base (B) is the sole component in a sample, but at low pH, N becomes protonated to yield the conjugate acid (BH^+). In the intermediate pH range, both B & BH^+ will be present in equilibrium. This can be described by an equilibrium expression [1] or a Henderson-Hasselbalch equation [2].



where **1:** pyridine R = R' = H
2: 2-picoline, R = Me, R' = H
3: 2,6-lutidine R = R' = Me

$$K_a = \frac{[H_3O^+][B]}{[HB^+]} \quad [1]$$

$$pH = pK_a + log\frac{[B]}{[HB^+]} \quad [2]$$

The equilibrium between B/BH^+ is rapid, so instead of 'seeing' two unique sets of resonances, a weighted, time-averaged chemical shift is observed. By varying the pH of a solution & noting the chemical shift, one can construct a titration curve. The equivalence point (*i.e.*, where [B] = [HB^+] & the molar fraction $x_B/x_{BH+} = 1$) can be extracted from the titration curve. This is where pH = pK_a.

$$\delta = \delta_{BH^+}x_{BH^+} + \delta_B x_B \quad [3]$$

$$x_B = \frac{\delta_{pH=1} - \delta}{\delta_{pH=1} - \delta_{pH=13}} \quad [4]$$

$$x_{BH^+} + x_B = 1 \quad [5]$$

Sample Preparation:

In groups of 4, obtain a nitrogen base from your TA. Prepare 5 mL of 1.0 M HCl & KOH solutions in D_2O & a 0.1 M KOH stock solution. These will be used to adjust the pH of your sample.

Prepare a sample containing 3 mL of a 0.25 M $N(Me)_4I$ in D_2O, 2 mL of 1.0 M HCl in D_2O, 12 mL of D_2O, and 0.25 mL of the provided nitrogen base in a wide mouth 50mL erlenmeyer flask. Add a clean stirring bar, place flask on a stir plate & introduce a pH meter. If the pH of your sample is >1, add HCl stock solution until the desired pH is reached. Transfer ~0.7 mL of this sample into an NMR tube & label it appropriately.

Slowly raise the sample pH with the KOH stock solution. Every 0.5-1 pH increments transfer a ~0.7 mL aliquot to an NMR tube & label it with the appropriate pH. At intermediate pH (<4, >7.5) NMR samples should be prepared with smaller (*e.g.*, 0.2) increments. As many samples are required for the experiment, after a spectrum is obtained, it can be returned to the flask if desired.

Data Collection:

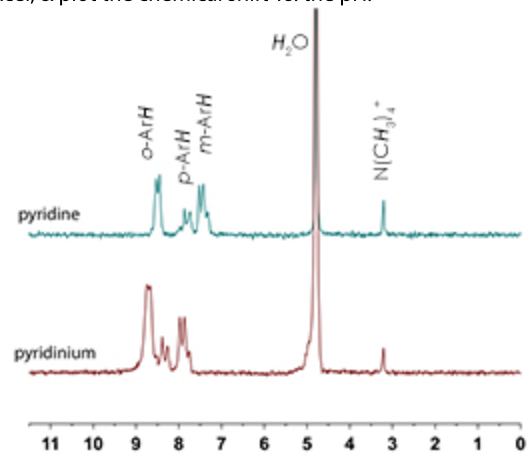
Measure a ^1H NMR spectrum for each sample (noting the appropriate pH) on the NMReady™ benchtop spectrometer using the following parameters:

SW = 12 ppm	ns = 32 scans
delay time = 0.5 sec	total time = 2 min

Save all the measured data to a USB drive and/or a network folder.

Data Treatment:

In MNova phase & baseline correct, making sure to apply the same correction to each spectrum. As the water peak is the predominate feature, increase the intensity until the pyridine/pyridinium resonances are fully visible. Reference each spectrum to $N(Me)_4I$ (δ = 3.207 ppm) & select a peak from each multiplet (*ortho-*, *meta-* & *para-*) that is well resolved. Tabulate these values in a graphing program, such as Excel, & plot the chemical shift *vs.* the pH.



Example Results for Pyridine:

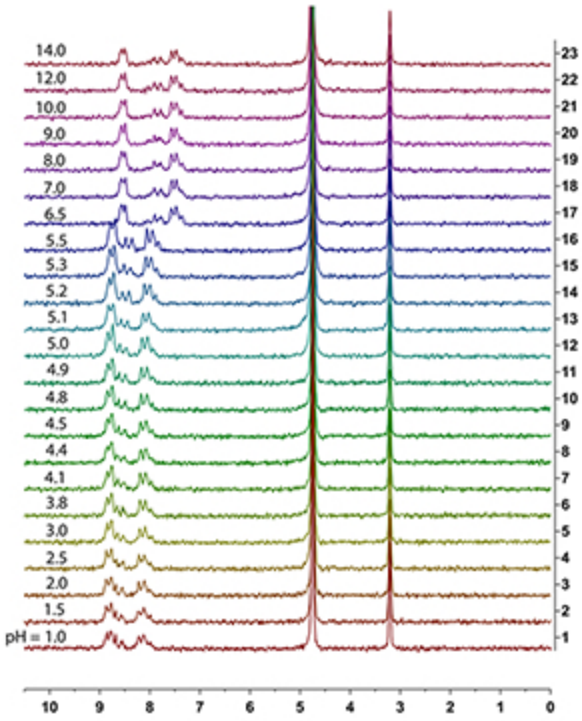


Figure 2: ^{1}H NMR Stacked plot of pyridine-pyridinium pH dependent equilibrium

If the chemical shift is displayed to 4 decimal places in MNova, the reference NMe_4^+ may not be exactly at 3.207 ppm. If not, correct it and apply this correction to the observed chemcial shift of the nitrogen base to ensure the chemical shifts are consistent throughout the pH range.

Table 1: Chemical shift of downfield *o-ArH* referenced to $N(Me)_4^+$ at 3.2070 ppm

Sample pH	δ of NMe_4 I (ppm)	Corrected to 3.207ppm	δ o-ArH downfield	δ o-ArH corrected (ppm)
1.0	3.2074	-0.0004	8.8679	8.8675
1.5	3.2074	-0.0004	8.8674	8.8670
2.0	3.2057	0.0013	8.8654	8.8667
2.5	3.2074	-0.0004	8.863	8.8626
3.0	3.2071	-0.0001	8.859	8.8589
3.8	3.2066	0.0004	8.8498	8.8502
4.1	3.2067	0.0003	8.8420	8.8423
4.4	3.2067	0.0003	8.8249	8.8252
4.5	3.2066	0.0004	8.8057	8.8061
4.8	3.2069	1E-04	8.7870	8.7871
4.9	3.2069	1E-04	8.7586	8.7587
5.0	3.2070	0	8.7300	8.7300
5.1	3.2069	1E-04	8.6941	8.6942
5.2	3.2067	0.0003	8.6808	8.6811
5.3	3.2074	-0.0004	8.6401	8.6397
5.5	3.2075	-0.0005	8.6210	8.6205
6.5	3.2070	0	8.5683	8.5683
7.0	3.2065	0.0005	8.5650	8.5655
8.0	3.2069	1E-04	8.5646	8.5647
9.0	3.2074	-0.0004	8.5690	8.5686
10.0	3.2067	0.0003	8.5673	8.5676
12.0	3.2072	-0.0002	8.5687	8.5685
14.0	3.2066	0.0004	8.5653	8.5657

The observed chemical shift can be plotted versus the pH to yield a titration curve:

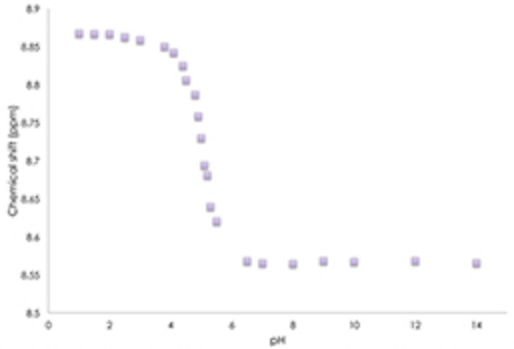


Figure 3: Observed ^{1}H NMR chemical shift of downfield *o*-ArH resonance relative to sample pH.

Discussion:

1) For one peak in each of the *ortho-*, *meta-*, *para-* resonances:
- **a)** In a graphing program, reference & correct chemical shifts.
- **b)** Plot chemical shift *vs.* pH.
- **c)** Find the equivalence point from each titration curves (*i.e.*, where $x_B = x_{BH+}$ & pH = pK_a).
- **d)** For each data point, calculate the mole fraction of pyridine (x_B) & pyridinium (x_{BH+}) using equation [4] & [5].
- **e)** Plot the log[x_B/x_{BH+}] *vs.* pH. According to the Henderson-Hasselbalch equation, the y-intercept will be the pK_a. How does this compare to the value you extracted from the titration curve?
- **f)** Compare the pK_a you determined via both methods with the literature value. Discuss both accuracy & sources of error in both methods.

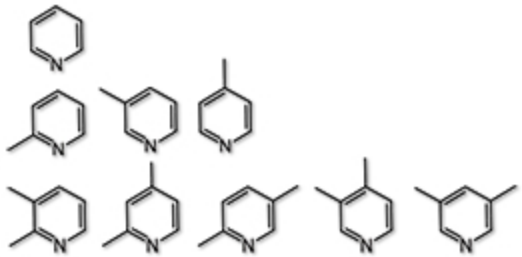
2) Which resonance (*o-*, *m-*, *p-*) is most effected by pH? Rationalize your observation.

3) Share your pK_a results with the rest of the class. Record the results they found so you have experimental data for the whole series of nitrogen bases.

4) Which nitrogen base is the most basic? The least? Why?

5) Is the pyridinium N-H resonance visible? Why or why not?

6) From your findings, predict the relative basicity of pyridine, 2- 3- & 4-picoline, & 2,3- 2,4- 2,5- 2,6-, 3,4- & 3,5-lutidine.



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References:

1) Handloser, C. S.; Chakrabarty, M. R., Mosher, M. W. *J. Chem. Educ.* **1973**, *50*, 510

2) Gift, A. D., Steward, S. M., Bokashanga, P. K. *J. Chem. Educ.* **2012**, *89*, 1458

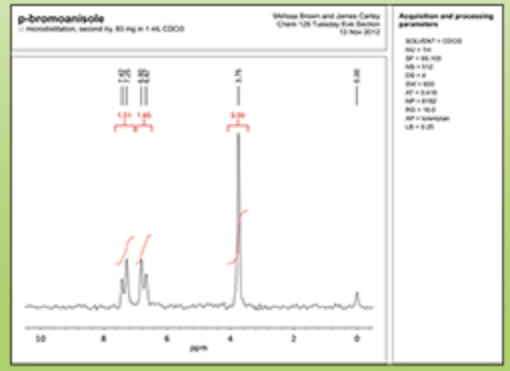
3) Berger, S., Braun, S. "200 & More NMR Experiments: A Practical Course" 2nd Ed. Wiley-VCH: Germany

4) Silverstein, R. M.; Webster. F. X.; Kiemle, D. J., "Spectrometer Identification of Organic Compounds" 7th Ed., John Wiley & Sons, Inc: USA, **2005**

5) Carey, F. A. "Organic Chemistry" 5th Ed., McGraw Hill: Toronto, **2003**

Data Accessibility:

NMReady outputs to a networked drive & has a print option. Students can process & print in third party software, like Mestrelab™, or use the NMReady directly. An example of data to be incorporated into a lab report processed and printed directly from the NMReady is presented below:



For additional ideas of how to incorporate the NMReady™ benchtop spectrometer into undergraduate laboratories please see:

1) Introduction to NMR Spectrometers: An Instrumental Analysis
2) Unknown Identification
3) Synthesis of Aspirin
4) Aldol Condensation

available at:

nanalysis.com/experiments.html