ThermoFisher SCIENTIFIC
The world leader in serving science

Thermo Fisher Scientific
Molecular Spectroscopy
525 Verona Rd, Madison, WI 53711
(608 276-6100
www.thermoscientific.com

Thermo SCIENTIFIC

picoSpin™ 45: Hydrolysis of Acetic Anhydride with Heavy Water (D_2O)

Dean Antic, Ph.D., Thermo Fisher Scientific, Boulder, CO, USA

1. Introduction

In a hydrolysis reaction, a chemical bond is broken by the addition water. Hydrolysis is typically carried out in the presence of a salt of a weak acid or weak base. Water autoionizes into hydroxyl ions (^{-}OH) and hydronium ions (H_3O^{+}) and acts as a source of a nucleophile and catalyzing acid, but it is also a weak acid and in most cases hydrolysis in water is to slow for the reaction to proceed without the addition of a strong acid. Hydrolysis of anhydrides are, however, often facile in the presence of water where only mild heating of the reaction mixture is necessary.

The hydrolysis of acetic anhydride (Ac_2O) to acetic acid (AcOH) serves as a model example of the hydrolysis reaction. Acetic anhydride rapidly hydrolyzes in the presence of water, alcohol and catalyzing acid, in this case water. We can monitor the evolution of the reaction using NMR by a modified in situ reaction monitoring whereby a single aliquot of the reaction mixture is injected into the RF coil of the NMR probe. In situ reaction monitoring by NMR has several requirements: 1) reactants and products must be soluble throughout the course of reaction, 2) signals undergoing change must be resolvable and 3) the rate of reaction must slower than the timescale of the NMR experiment.

In addition to its applications in the determination of static molecular structures, many NMR experiments are performed to monitor the growth and evolution of resonance signals undergoing dynamic change. An example of a time-dependent process is a chemical reaction. During a reaction, resonance signals shift position, coalesce, grow and diminish in intensity. Tracking and extracting chemically relevant information by NMR requires that the timescale of the dynamic process be slower than the so-called NMR timescale. The NMR timescale finds it basis in the uncertainty principle, where the width of resonance, $\Delta\nu$, at a given frequency is measurable as a distinct sharp line if the lifetime, $1/\tau$, of the state is long.

$$\Delta\nu = h/(2\pi\tau)$$

As lifetime of the resonance shortens, broadening of the signal occurs. This is referred to as lifetime broadening. Lifetime broadening is evident in the broad resonances observed for

rapidly exchanging labile protons, such as in alcohols. The minimum timescale requirement for averaging two closely spaced resonances is the reciprocal of the difference of the peaks. Otherwise, the signals begin to coalesce.

2. Purpose

In this experiment, we monitor changes occurring during the course of a simple reaction, the hydrolysis of acetic anhydride with heavy water (D_2O) by a modified in situ reaction monitoring technique. We also take advantage of isotopic substitution to suppress an otherwise large proton signal in the NMR spectrum originating from the reactant/solvent H_2O. Isotopic substitution does not alter the potential energy surface along the reaction coordinate, but it will affect the rate of reaction by changing the enthalpy of activation.

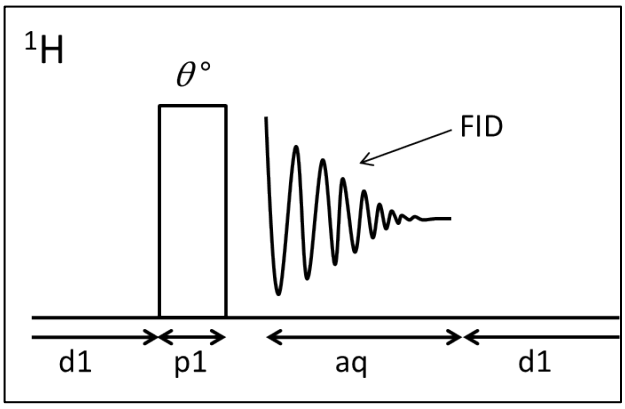
In water, hydrolysis converts acetic anhydride to acetic acid, a carboxylic acid. Acetic acid is a weak acid that partially dissociates to hydronium and acetate ions. Using NMR, we can follow the reaction by monitoring the relative sizes of the acetyl resonance of the reactant, acetic anhydride, as it is consumed, while simultaneously observing the growth of the acetyl signal from the acetic acid product. Both acetyl signals have similar chemical shifts, 2.26 and 2.10 ppm, respectively, but are easily resolved using the Thermo Scientific™ picoSpin™ 45 ^{1}H NMR spectrometer. Here we will learn the basic skills of monitoring the course of a chemical reaction as it evolves within the RF coil of the NMR spectrometer. This in situ approach can be applied to other liquid phase reactions.

3. Literature

a. Binder, D. A.; Ellason, R.; Axtell, D. D., Kinetic hydrogen isotope effect, *J. Chem. Educ.*, **1986**, *63*, 536.
b. Gold, V. The Hydrolysis of Acetic Anhydride, *Trans. Faraday Soc.,* **1948**, *44*, 506-518.
c. Seoud, O.A., Bazito, R. C. and Sumodjo, P. T., Kinetic Solvent Isotope Effect: A Simple, Multipurpose Physical Chemistry Experiment, *J. Chem. Educ.*, **1997**, *74*, 562
d. Lowry, T.H.; Richardson, K.S. *Mechanism and Theory in Organic Chemistry*, 3rd Ed., Harper and Row, 1987 pp 232-244. Carey, F.A.; Sundberg, R.J. *Advanced Organic Chemistry Part A*, 2nd Ed., Plenum Press, pp 190-194.

4. Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to allow the acquisition of an FID at a desired time step.



Sequence: d1–[θ°–aq–d1]$_{ns}$
θ°: Pulse rotation angle (flip angle)
FID: Free induction decay
d1: Recycle delay (µs) for spin-lattice relaxation
p1: R.F. transmitter pulse length (µs)
aq: Acquisition time (ms)
ns: # of scans (individual FIDs)

5. Procedure & Acquisition

Time requirements: 45 min

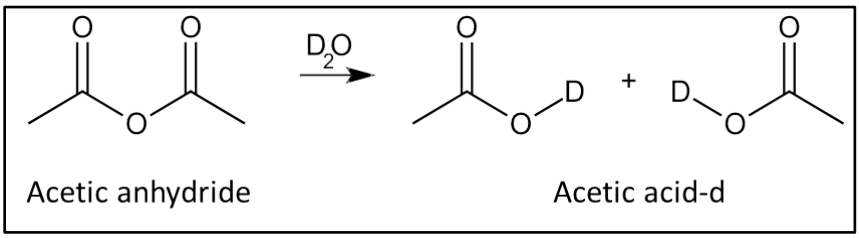
Sample: 2% (v/v) acetic anhydride in deuterium oxide (D_2O)

Difficulty: Easy

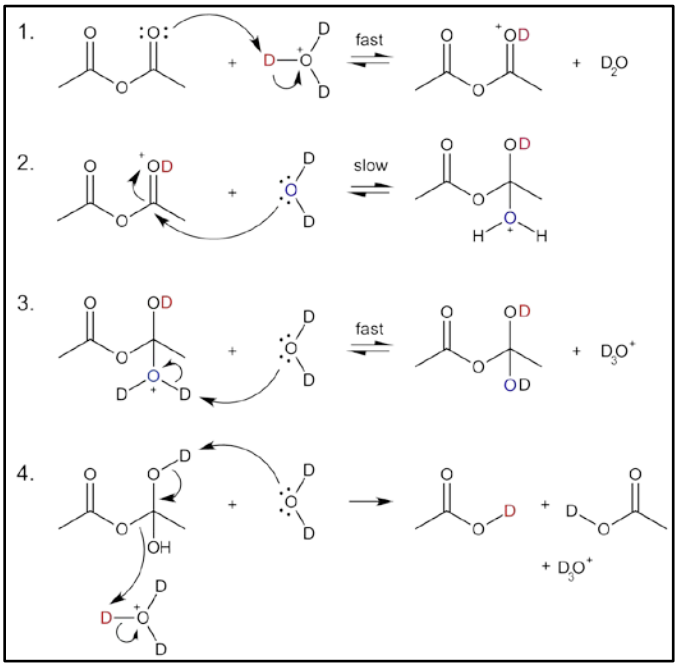
Equipment/material:

- Thermo Scientific™ picoSpin™ 80
- Acetic anhydride ($C_4H_6O_3$)
- Deuterium oxide (D_2O)
- 1 mL vial with PTFE cap liner
- 1 mL polypropylene syringes
- 22 gauge blunt-tip dispensing needles
- Mnova NMR Processing Suite
- picoSpin accessory kit
  - Drain tube assembly
  - Syringe port adapter
  - Port Plug

Reaction:



Mechanism:



Physical data:

Substance	FW (g/mol)	Quantity	MP (°C)	BP (°C)	Density (g/mL)
acetic anhydride	102.09	10 µL	-73.1	139.8	1.08
deuterium oxide (D_2O)	130.19	490 µL	3.8	101	1.11
acetic acid-d	61.06	product	16	118	1.05

Experimental procedure:

The general procedure for sample analysis using a picoSpin NMR spectrometer is as follows:

Shim

- Prior to beginning the reaction, ensure the NMR spectrometer is shimmed and ready to accept samples.

Pre-reaction preparation

- Displace the shim fluid from the picoSpin capillary cartridge with air.
- Flush the cartridge with 0.1 mL of chloroform-d or acetone-d_6, then displace the solvent with an air push.

- Set up the onePulse script according to the parameters listed in the *Pulse Script* table below.

Reaction preparation

- To 0.5 mL of D_2O in a 2 mL vial add 0.01 mL of acetic anhydride.
- Cap the vial and shake it for a few seconds (alternatively, use a vortex mixer).
- The reaction begins as soon as the reactions are mixed, so be prepared to inject the sample soon after mixing.

Injection

- Using a 1 mL disposable polypropylene syringe fitted with a 1.5" long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of the reaction mixture.
- Inject about half the sample. Ensure all air bubbles have been displaced for the cartridge by examining the drain tube.
- Seal both the inlet and outlet ports with PEEK plugs.

Acquire

- Execute the onePulse script according to the values in the table of parameters provided.
- Once the onePulse script has finished and the reaction is completed, prepare the cartridge for the next user by displacing the reaction sample from the cartridge according to the following protocol: air, solvent, air.

Pulse Script: onePulse

Parameter	Value
tx frequency (tx)	proton Larmor frequency (MHz)
auto tx	✓
auto tx offset (o1)	0 Hz
scans (ns)	30
pulse length (p1)	Instrument specific 90° pulse length
acquisition time (aq)	750 ms
rx recovery delay (r1)	500 µs
T1 recycle delay (d1)	60 s
bandwidth (bw)	4 kHz
post-filter atten. (pfa)	10 (11)[a]
phase correction (ph)	0 degrees (or any value)
exp. filter (LB)	0 Hz
max plot points	400
max time to plot	250 ms
min freq. to plot	-400 Hz
max freq. to plot	+800 Hz
zero filling (zf)	8192
align-avg. data	✓
live plot	✓

JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default pfa values

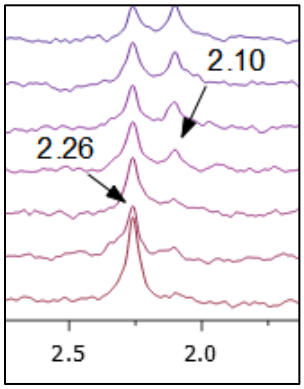
6. Processing

After data acquisition, spectra need to be processed. Download and open the experimental JCAMP spectrum file by importing it into Mnova. The Free Induction Decay (FID) will undergo automatic Fourier transformation and a spectrum will be displayed.

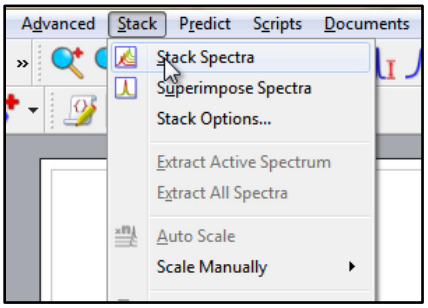
To each spectrum, apply the following processing steps using the given settings:

Function	Value
Zero-filling (zf) & Linear Predict (LP)	16 k
Forward predict (FP)	From aq → 16 k
Backward predict (BP)	From -2 → 0
Phase Correction (PH)	PH0: Manually adjust
	PH1: 0
Apodization	
Exponential (LB)	0.6 Hz
First Point	0.5
Shift reference (CS)	Manually reference
Peak Picking (pp)	Manually Select Peaks
Integration (I)	Automatic Selection
Multiplet Analysis (J)	-

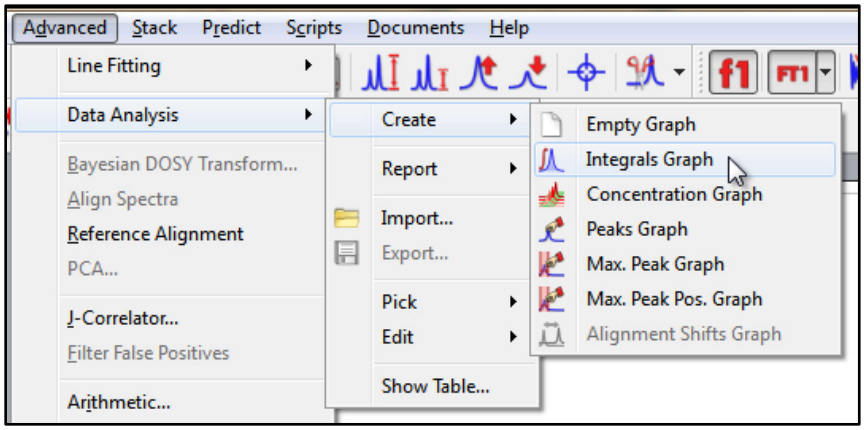
- Import each data file into the same workspace in Mnova. There should be 30 NMR spectra displayed in the 'Pages' view in Mnova.
- Highlight all spectra and process them simultaneously, including Ph0 phase correction.
- Manually shift reference each spectrum using Mnova's TMS tool by assigning a chemical values of 2.10 ppm for the right most signal, or 2.26 just to the left of this signal. It is important that the spectra be properly referenced.



- While all spectra are highlighted, navigate to the menu option '/Stack/Stack Spectra' and select it (or click the 'stack spectra' icon on the Stacked NMR toolbar). A new stacked spectrum page will appear.



- Zoom into the stacked spectra to display a chemical shift range from about -0.5 to 6.0 ppm.
- Select the '/Advanced/Data Analysis/Create/Integrals Graph'. An 'integration' icon will appear.



- Highlight the signal at 2.26 ppm and an integrated peak area vs. data array time data plot will appear.
  - Select the region containing the Ac_2O signal for integration and a small data plot will appear above the stacked spectra.
  - In the arrayed data column of the Data Analysis window click the X(I) 'Model' heading to change the X(I) function to "I-1" and choose the units of "min"; click 'OK'.
- Re-phase the spectra and repeat the data analysis for the second peak at 2.26 ppm.
- Highlight the signal at 2.10 ppm and an integrated peak area vs. data array time data plot will appear.
  - Select the 'Create Integrals' option from the icon in the upper left hand corner of the Data Analysis window to create a second integrals plot. Select the region containing the AcOD signal for integration and a small data plot will appear above the stacked spectra.
  - In the arrayed data column of the of the Data Analysis window click the X(I) 'Model' heading to change the X(I) function to "I-1" and choose the units of "min"; click 'OK'.
- The plots can be repositioned so as not to be overlapping. Formatting options are available by double clicking a data plot.

7. Results

The predicted ^{1}H NMR spectrum for equi-molar quantities of the reactant acetic anhydride (Ac_2O) and product acetic acid-d (AcOD) is presented in Figure 1. The spectrum contains only two signals, one each from the acetyl group protons [-C(=O)CH_3] arising from both the reactant and product. The change from acid anhydride to carboxylic acid results in a small upfield shift of the acetyl signal making each signal group easily resolvable.

Acetic anhydride is an acid anhydride. The ^{1}H NMR spectrum of neat Ac_2O is characterized by a single upfield (low frequency) chemical shift of the acetyl group protons appearing at 2.26 ppm and an integration value of 6, due to two CH_3 groups contributing to the signal. It appears as a singlet in the proton spectrum because of the lack of neighboring protons.

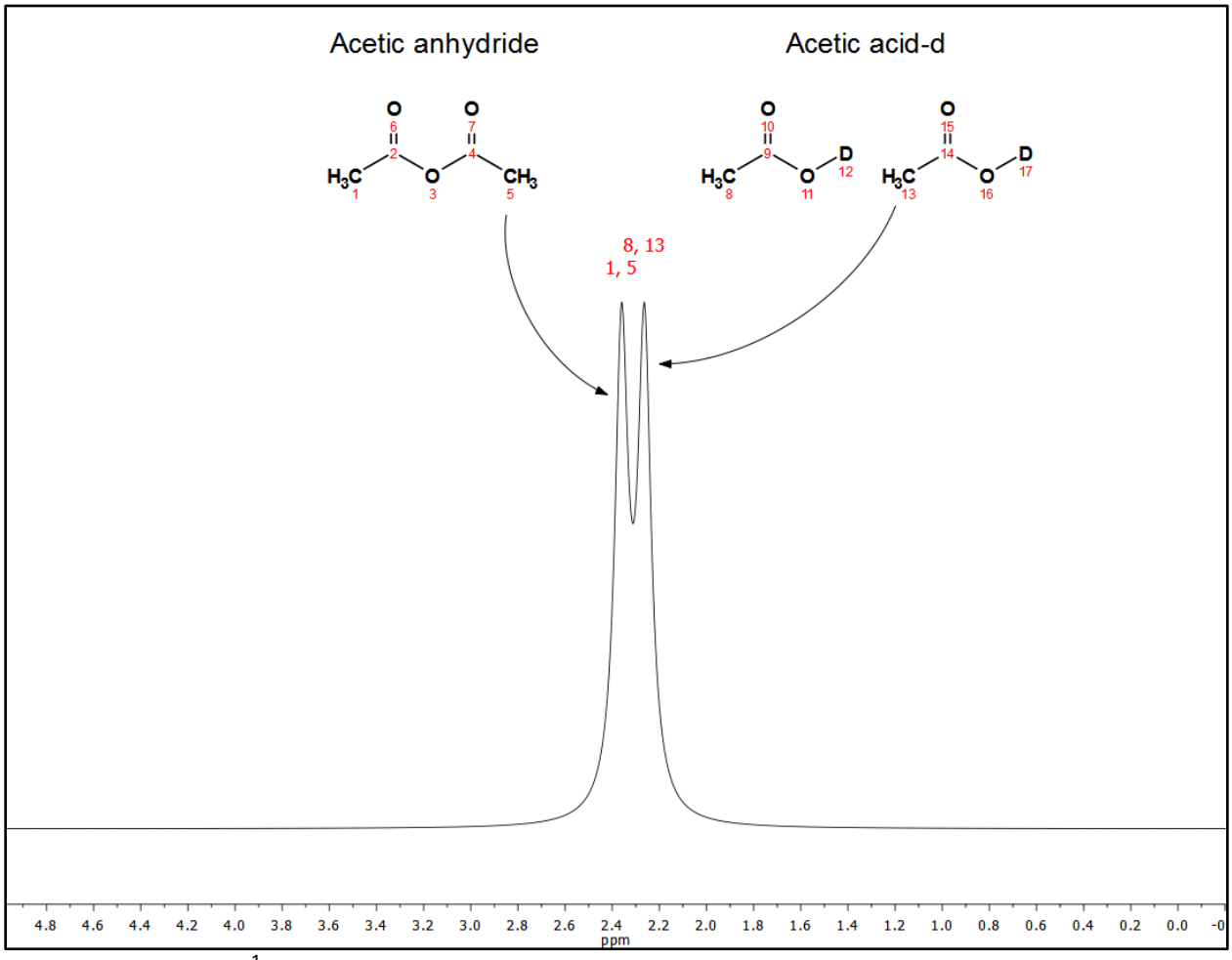


Figure 1. Predicted ^{1}H NMR (45 MHz) spectrum of acetic anhydride and acetic acid-d.

Acetic acid contains a carboxylic acid. Proton NMR spectra of neat carboxylic acids are identified by a characteristic downfield (high frequency) chemical shift of the acid proton. The acidic nature of carboxylic acid protons make them strongly deshielded, with signals typically appearing between 11-12 ppm. Acidic protons also experience intramolecular hydrogen bonding and exchange rapidly, and rapidly exchanging protons tend to result in narrow signals. Adding a drop of D_2O (heavy water) to the sample causes the peak to disappear; this is evidence for the presence of a carboxylic acid, but then labile protons from alcohols, amines, thiols, phenols and enols will also exhibit this exchange behavior. Similarly, acidic protons experience intermolecular exchange with labile protons from other compounds, such as water, causing the signal to broaden and shift upfield (low frequency), closer to the labile proton chemical shift.

In this reaction, we hydrolyze Ac_2O in the presence of D_2O, thus the proton spectrum of the product AcOD will only contain one signal due to the acetyl group protons. The signal

appears slightly upfield of the Ac_2O signal at 2.10 ppm and will have an integration value of 6 since 2 moles of AcOD are produced from 1 mole of Ac_2O.

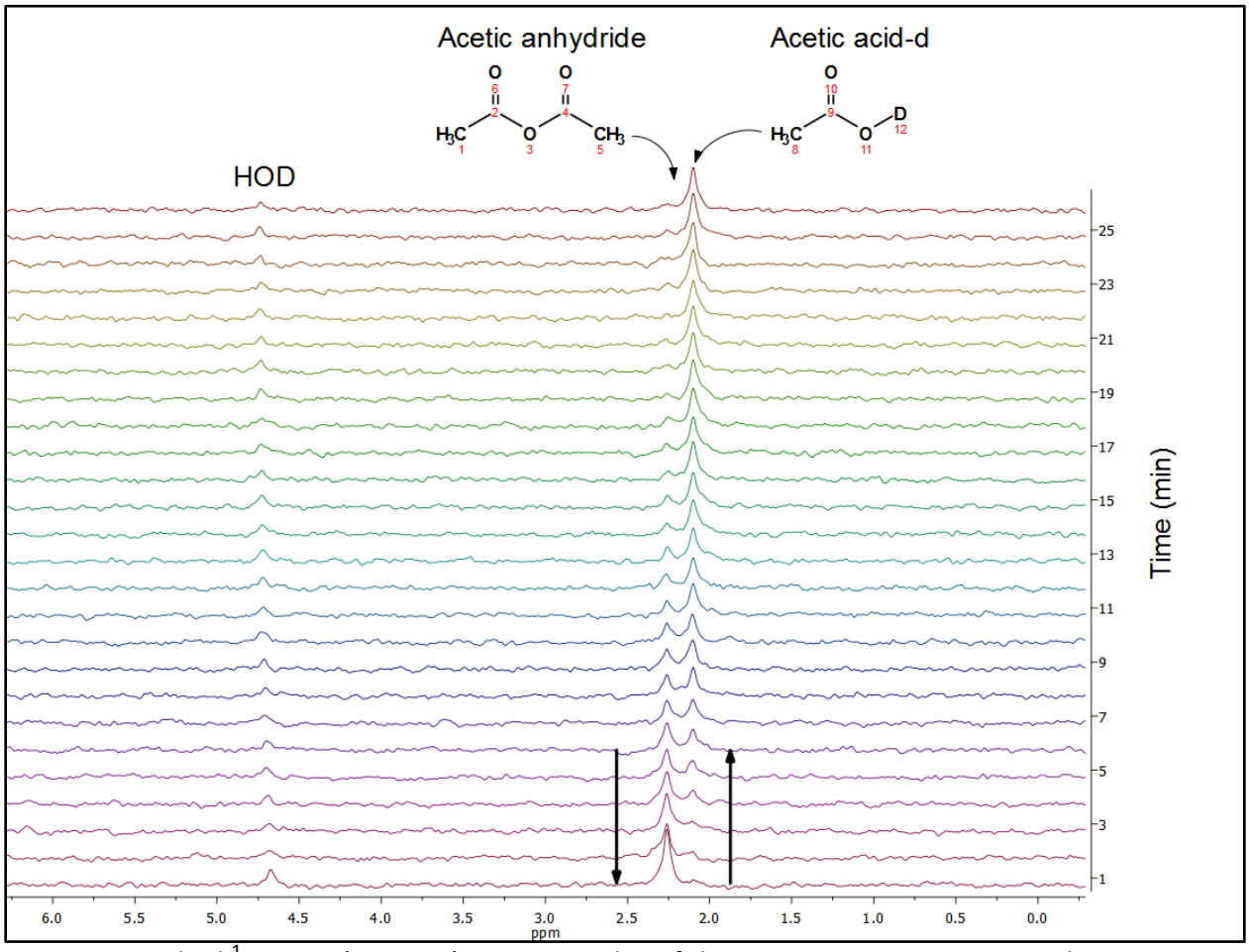


Figure 2. Stacked ^{1}H NMR (45 MHZ) spectrum plot of the reaction mixture acquired at 1-minutes intervals.

Initially, at t = 0 min, the NMR spectrum of the reaction mixture (Figure 2) contains two signals, one at 4.6 ppm and is assigned to a residual HOD water signal, and one arising from the reactant Ac_2O appearing at 2.26 ppm. As the reaction proceeds, the Ac_2O diminishes in intensity as a second signal at 2.10 ppm develops. The new signal is due to the product AcOD. At $t \approx 7$ min, the intensity of the two acetyl signals are nearly identical and as the mixture continues to react, the reactant signal from Ac_2O continues to shrink while the product signal grows. At $t \approx 30$ min the reaction is nearly completed and the NMR spectrum is dominated by the product signal.

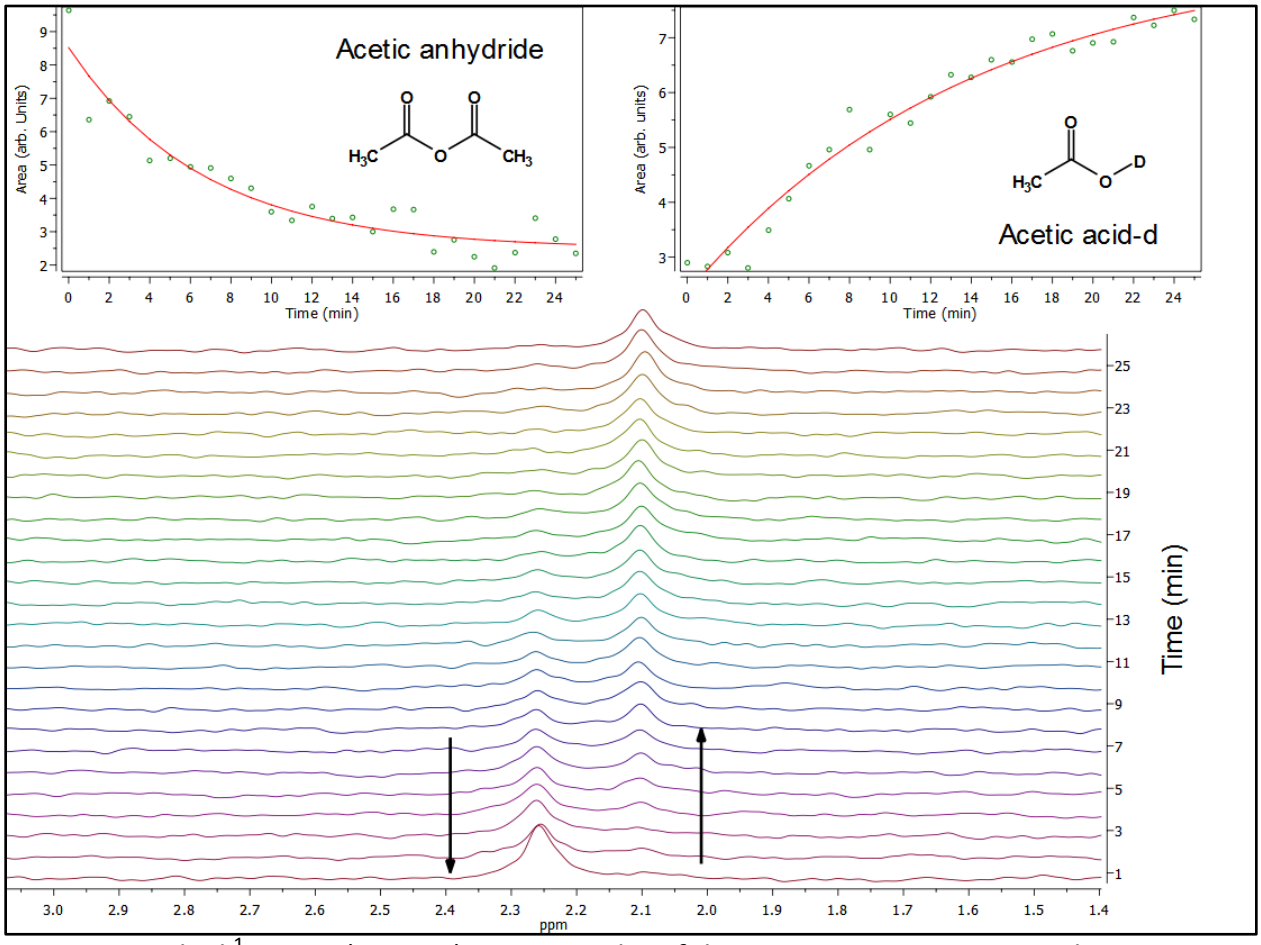


Figure 3. Stacked ^{1}H NMR (45 MHz) spectrum plot of the reaction mixture acquired at 1-minute intervals. Data plots displaying area plotted against time for the 2.26 ppm and 2.10 ppm signals.

Integrating the peak areas of the reactant produces pseudo-first order rate curves as seen in Figure 3. In this figure, the integrated peak areas for the AC_2O and AcOH signals are plotted as a function of time. The procedure for creating these data plots using Mnova is provided in the Processing section. Since the reaction begins almost as soon as the reactants are mixed, the elapsed time from mixing the reactants to injection to initiating data acquisition should be kept at a minimum. From the intensity vs. time plots, we see the Ac_2O signal diminishes roughly at the same rate as the AcOH signal grows.

8. Comments

Initially acetic anhydride is insoluble in water. Vigorously shake the reaction vial for several seconds to solubilize the reactant, or use a vortex mixer. Acetic anhydride is soluble in water to only ~2.6%, so it is important not to increase the relative volumes.

Table 1. ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
1-3	acetic acid-d	DO-C(=O)CH_3	2.10	3 H	Singlet
1-3	acetic anhydride	$CO_2(CH_3)_2$	2.26	6 H	Singlet
2,3	residual water	HOD	4.65	1 H	Singlet

9. Own Observations