NMR

picoSpin Example Lesson Plans

269-307000 Revision A April 2014

Thermo SCIENTIFIC

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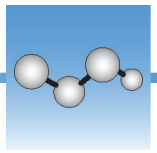
WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

Contents



Introduction

Purchasing and installing a new NMR spectrometer for your organic chemistry teaching laboratory can be daunting, especially if you are the only member of the faculty that has any previous experience with NMR. Time may be short before the beginning of the next semester and you are the one that has to turn your new spectrometer into a positive learning experience for the students that depend on you for their future education. There may be a lot of uncertainty when faced with integrating an NMR instrument into your student's workflow, such as "how much sample do they need to get a good NMR spectrum quickly?", or "what experiments are best suited for an undergraduate teaching environment that will also make great candidates for easy and useful NMR interpretation?"

This set of lesson plans aims to address both of the points above, and many more, to help ease the process and effort of offering valuable hands-on experience with NMR to your students with the Thermo Scientific™ picoSpin™ 45 or Thermo Scientific™ picoSpin™ 80 NMR spectrometer. In the booklet, you will find a number of distinct lesson plans that you can directly implement into your laboratory. They are all refined specifically for the undergraduate teaching laboratory, and some are already commonly found in undergraduate teaching programs.

Each laboratory/lesson plan contains: full instructions regarding experimental aspects, with a comprehensive listing of chemicals and equipment needed; details for NMR data acquisition, processing, and analysis; leading finally to a full interpretation of the results that will enrich your student's appreciation of science. Some of the lesson plans are adapted for both 45 MHz and 80 MHz, and some have additional notes for the instructor to aid in implementation.

If you would like to contribute to a future edition of this lesson plan guide, or if you have feedback as to how we may improve it, please contact Mark Dixon by
e-mail: mark.dixon@thermofisher.com

Safety Precautions

CAUTION Avoid personal injury.

- Wear eye protection at all times when handling liquid chemicals
- Do not breathe hazardous vapors
- Avoid skin contact with hazardous liquids and vapors
- Eliminate ignition sources and prevent significant waste volume buildup

WARNING Avoid personal injury. Use a fume hood, if necessary, and wear appropriate protective equipment. Ejecting air bubbles from the syringe may eject a small volume of liquid that could be hazardous.

WARNING Avoid personal injury.

- Needles and syringes should be considered regulated waste regardless of use
- Follow your local EH&S guidelines for disposal
- Never throw these items into the regular trash or dumpsters

An Introduction to Quantum Mechanics and Multiplet Splitting in NMR Spectra

Introduction

Quantum mechanics is an incredibly important theory in chemistry that effectively describes the behavior of molecules, atoms, and other subatomic particles. Unfortunately, the complexity of the mathematics required to describe quantum mechanics, and the abstract nature of the particles being described, often makes it an extremely difficult topic to communicate effectively.

Nuclear magnetic resonance (NMR) spectroscopy, arguably the most important analytical technique in organic chemistry, is similarly a challenging topic to introduce to students. The abstract concepts, complex mathematics, and sometimes dizzying rotation taking place are difficult even for some faculty. As a result, many students in undergraduate chemistry courses are never exposed to these two important topics.

Purpose

In this classroom demonstration we will simplify the explanation and make the abstract concepts more concrete by combining quantum mechanics and NMR spectroscopy in one demonstration. Although NMR spectra can be obtained using a number of different nuclei, we will focus on ^{1}H or proton NMR.

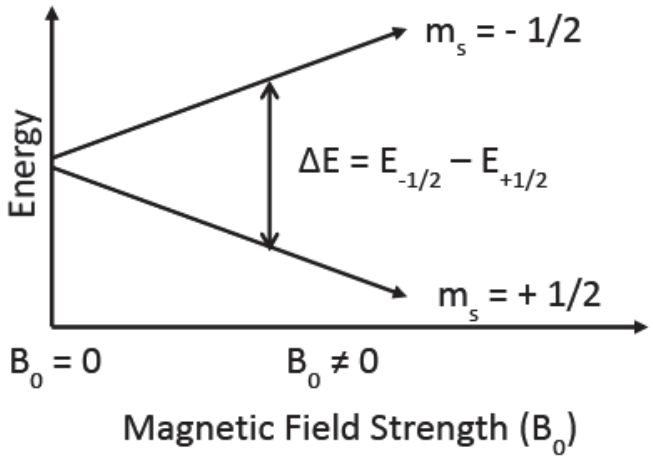
Shortly before the discovery of quantum mechanics it was discovered that the Zeeman Effect and other fine structure spectral lines could be explained if the electrons were spinning about their own axis. This property, known as spin angular momentum was eventually extended to protons as well. The reasoning, and mathematical explanation of this spin is beyond the scope of this demonstration, but suffice it to say that it has been well studied and now represents the fourth quantum number m_s (the first three are the principal, orbital, and magnetic quantum numbers).

Because protons and electrons also have a charge associated with them, this intrinsic spin causes the particle to behave as a very small magnet. (A full discussion of induction and induced magnetic fields is outside the scope of this demonstration; please consult a physics text for a detailed description of this concept.) These particle-sized magnets are randomly

oriented in most instances, but can be forced to adopt one of only two orientations by placing the particles inside a strong magnetic field. In this situation, the particles orient themselves along the axis of the applied magnetic field $\mathbf{B}_0$ with the particle's magnetic field $\mathbf{B}_p$ oriented either with, or against the applied field. These two states, referred to as "spin up," and "spin down" have, as you might expect, two different energy levels as seen in Figure 1. As all matter prefers to be in the lowest energy level possible, more particles adopt the lower energy spin up orientation with the particle's magnetic field aligned with the applied magnetic field $\mathbf{B}_0$ than adopt the higher energy spin down state. This population difference is what NMR spectroscopy, similar to other optical spectroscopies (UV-Vis, Infra-red, etc.) uses to generate an NMR signal.

Figure 1

The effect of an external magnetic field B_0 on the spin orientation of the nucleus. The two energy levels are populated according to the Boltzmann distribution



The fact that every chemically different proton (for ^{1}H NMR) is in a slightly different magnetic environment, means that the energy level's available to those nuclei are also slightly different. These small differences in energy from one proton to another allow the NMR signal to become (with sufficient instrumental resolution) an NMR spectrum with a different peak for each chemically unique proton.

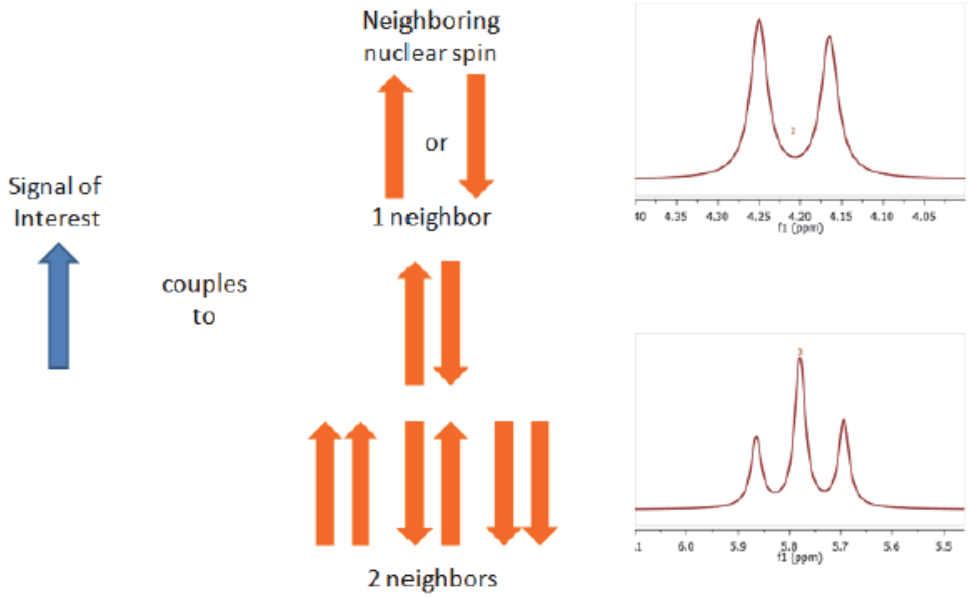
The displacement of NMR peaks along the x-axis is known as chemical shift and reveals information about what functional groups are present near the proton responsible for a given signal. In addition, the relative integration of the signals allow NMR spectra to quantify the number of protons that are responsible for generating a given signal, and even to quantify the relative concentration of sample mixtures.

The final piece of information that an NMR spectrum tells us is the multiplicity of the signal and this is what we will focus on in today's demonstration. Multiplicity is the splitting of a given NMR spectral signal into a tightly clustered series of peaks based on the number of protons within a given distance from the proton responsible for the signal (typically three bonds between interacting nuclei).

If we once again consider the spinning nuclei, the magnetic field produced by the spin causes small changes in the electron distribution of its bonds. These in turn effect the electron distribution of neighboring bonds, which affect the neighboring nuclei. This process is known as spin-spin coupling and is typically observable out to three bond lengths. Longer range effects are observable, but are beyond the scope of this demonstration. As both neighboring protons can be oriented either spin up or spin down, their effects on the electron cloud, and therefore on each other, will depend on all the possible spin-spin combinations. In the simplest case, when there are only two protons interacting with each other, there exist four total combinations (up-up, up-down, down-up, and down-down), but only two combinations with unique energies (up-up and down-down have identical energies, as do up-down and down-up). This causes the original NMR signal to split in two, and is known as a "doublet." A proton with two neighboring protons would have four unique combinations but only three unique energies, up-up-up, up-up-down (up-down-up is a unique combination but has the same energy as up-up-down), and up-down-down. Because there are twice as many unique combinations for the up-up-down energy level, the height of that peak will be approximately double that of the outer peaks as shown in Figure 2.

Figure 2

Spin-spin coupling to form multiplets. The formation of a doublet is created by the two unique coupling combinations between the signal nuclei (in blue) and the single neighbor (which can be up or down). The formation of a triplet results from the four unique coupling combinations between the signal nuclei and two neighboring nuclei. Although there are four unique combinations, two of the combinations have the same energy associated with them limiting the number of peaks to three.



As mentioned above, the displacement of NMR signals along the *x*-axis of an NMR spectrum is dependent upon the magnetic field strength of the instrument, however, the distance between peaks making up a multiplet are not field strength dependent. This is in agreement with the multiplet being a result of the spin orientation of the nucleus on the electron cloud, an interaction that is independent of the field strength.

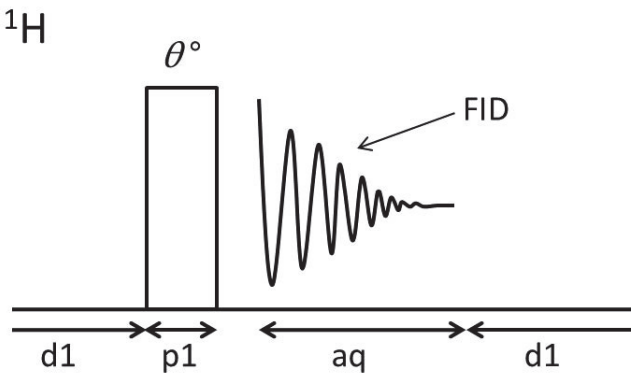
By measuring the spacing of the multiplet peaks we can directly observe the effect these two quantized nuclear spin states (spin up or spin down) have on their environment.

Safety Precautions

NOTICE Be sure that all persons operating this system read the site and safety manual first.

Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



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 tim (ms)
ns: # of scans (individual FIDs)

Procedures and Analysis

Equipment/Materials

- picoSpin 45 or picoSpin 80
- 1 mL polypropylene syringe
- 22 gauge blunt tip dispensing needle
- Syringe port adapter and picoSpin drain tube assembly
- 0.5 mL Ethyl Acetate

Experimental Procedure

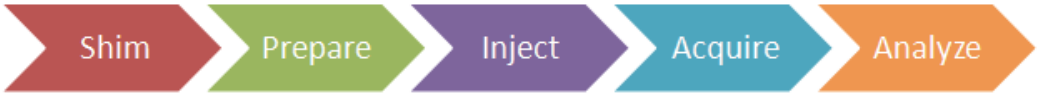
1. Install the NMR spectrometer in a safe location that is convenient for the demonstration.
2. Allow the instrument to warm up and shim the instrument using water.

   Consult the picoSpin *Installation and Setup* guide provided with your spectrometer for details.
3. Attach the spectrometer drain tube assembly.
4. Using the syringe, draw up and then inject ethyl acetate into the spectrometer.

   Take care not to inject air bubbles.

Instrumental Procedure

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



1. Navigate to the spectrometer's **Run > onePulse** script.

Pulse Script: onePulse

Parameter	Value
tx frequency (**tx**)	proton Larmor frequency (MHz)
scans (**ns**)	16
pulse length (**pl**)	Instrument specific 90° pulse length
acquisition time (**aq**)	750 ms
T1 recycle delay (**d1**)	10 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	-200 Hz
max freq. to plot	+1000 Hz
zero filling (**zf**)	8192

Parameter	Value
align-avg. data	✓
live plot	✓
JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default **pfa** values

2. Acquire a 16 scan averaged spectrum.

   This will take one minute and 45 seconds and is a good time to introduce the guided inquiries questions.

Processing

Download the experimental JCAMP spectra files and open them by importing 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:

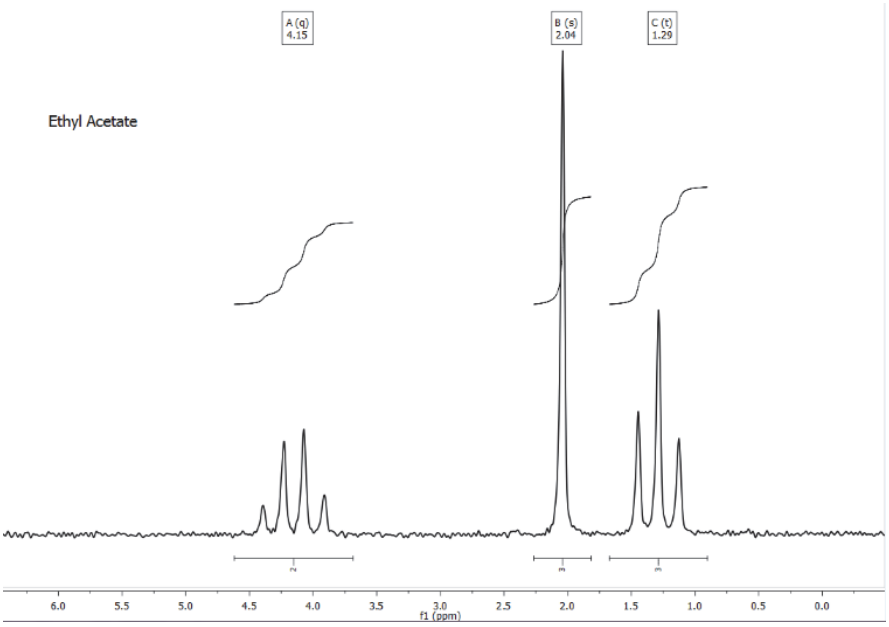
Function	Value
Zero-filling (zf) & Linear Predict (LP)	16 k
Forward predict (FP)	From aq → 16 k
Backward predict (BP)	From -2 → 0
Phase Correction (PH)	PH0: Manually adjust
	PH1: 0
Apodization	
Exponential (LB)	0.6 Hz
First Point	0.5
Shift reference (CS)	Manually reference
Peak Picking (pp)	Manually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

1. Import each data file into the same workspace in Mnova.
2. Manually apply Ph0 phase correction to each spectrum.
3. Manually shift reference each spectrum using Mnova's TMS tool.

4. Assign the TMS signal (0 ppm) or $CHCl_3$ signal (7.24 ppm), whichever is present.
5. Identify and assign each signal in the spectra.
6. Save the Mnova document, print each spectrum and paste into your lab notebook.

Results

1. Measure the peak spacing of the triplet.
2. Using the relationship $\Delta E = h\nu$ where ΔE is the energy difference observed, h is Planck's constant, and ν is the peak separation in Hz, calculate the energy difference of the interaction between the spin up and spin down protons.
3. Repeat previous two steps for the quartet.



Guided Inquiry Prequestions

- Will the individual peak spacing within a given multiplet be identical? Why or why not?
- Do you expect the ΔE from the triplet and the quartet to be different? Why or why not?

Guided Inquiry Post-Questions

- Assign all the peaks in your NMR spectrum and attach to your lab notebook.
- Was the individual peak spacing within a given multiplet identical? Why or why not?
- What were the values you calculate for $\Delta E_{triplet}$ and $\Delta E_{quartet}$?
- Were the values of $\Delta E_{triplet}$ and $\Delta E_{quartet}$ identical? Explain.

Own Observations

Ordering Information

To reorder this lesson plan from the *picoSpin NMR Spectroscopy: Example Lesson Plans* set, please refer to document order number: "**LP52588_E 05/14M** - picoSpin Lesson Plan #1 - Quantum Mechanics and Multiplet Splitting in NMR"

For U.S. Technical Support, please contact:

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For International Support, please contact:

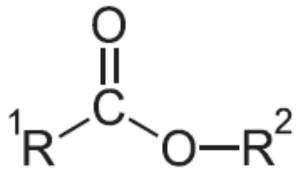
Thermo Fisher Scientific
Telephone: +1 608 273 5017
E-mail: support.madison@thermofisher.com

Note Please have the instrument serial number available when you contact us.

Fisher Esterification Synthesis of Isopentyl Acetate (Banana oil): picoSpin 45

Introduction

Esters are a class of compounds found widely in nature. Low molecular weight esters tend to have characteristic flavors and pleasant odors that are most often associated with essential oils, even though essential oils often are a complex mixture of natural compounds.



The ^{1}R and ^{2}R group side chains can be linear or branch-chain aliphatic or aromatic groups and they can be the same or dissimilar groups.

The ester functional group can be synthesized by many methods. The simplest approach is Fisher esterification. By this method, esters are produced by refluxing a carboxylic acid and an alcohol in the presence of a concentrated acid catalyst. To exploit Le Chatelier's principle and shift the position of the equilibrium to the right we add an excessive of one of the reactants to the reaction mixture. Naturally, we choose the least expensive reactant, which, in this case, is the carboxylic acid. The reaction mechanism involves initial protonation of the carboxyl group, nucleophilic attack by the hydroxyl, proton transfer, and loss of water followed by loss of the catalyzing acid to produce the ester. The process is thermodynamically controlled yielding the most stable ester product. Typically, only primary and secondary alcohols are used in the Fisher method since tertiary alcohols are prone to elimination. In this lab, you will perform a Fisher esterification to synthesize isopentyl acetate from isopentyl alcohol and acetic acid.

Purpose

The purpose of this experiment is to synthesize isopentyl acetate (3-methylbutyl acetate) via an esterification reaction between acetic acid and isopentyl alcohol (3-methylbutanol), using concentrated sulfuric acid as a catalyst. The product will be washed, distilled, then characterized using NMR spectroscopy.

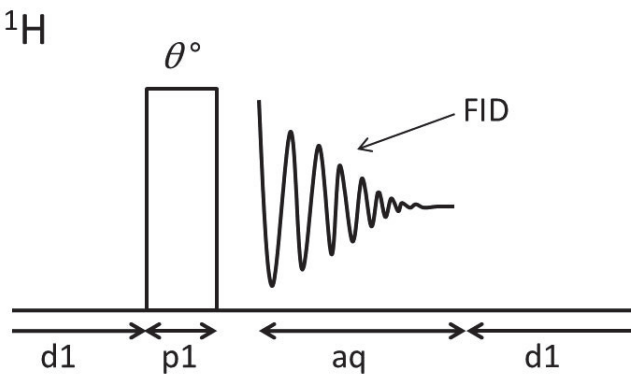
Literature

Adapted from: Gokel, H. D.; Durst, G. W. *Experimental Organic Chemistry*; McGraw-Hill, New York, **1980**; pp 344-348.

Weast, Robert C., ed. *CRC Handbook of Chemistry and Physics*. 70th ed. Boca Raton, FL: CRC Press, Inc., **1990**.

Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



Sequence: d1-[$\theta°$-aq-d1]$_{ns}$
$\theta°$: 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 tim (ms)
ns: # of scans (individual FIDs)

Procedures and Analysis

Time requirements: 3 to 3.5 hours
Difficulty: Moderate
Sample: Acetic acid, isopentyl alcohol, isopentyl acetate

Equipment/materials:

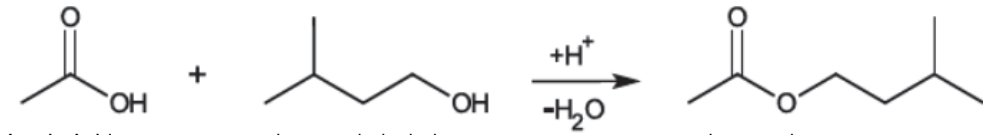
- picoSpin 45
- NMR processing software (Mnova™)
- Ring stand
- Iron ring
- Ice bath
- Syringe filter (optional filter)

- Sand bath (or electric mantle)
- Boiling chips
- Separatory funnel
- Thermometer
- Clamps (flask or Keck)
- Simple distillation apparatus
  - 100 mL round bottom flask
  - 25 mL Erlenmeyer flask
  - Condenser
  - Three-way adapter
  - Vacuum adapter
- Thermometer adapter
- Syringe port
- Port Plug
- Tubing
- Reflux distillation apparatus
  - 50 mL round bottom flask
  - Condenser
  - Drying tube
- picoSpin accessory kit
  - 1 mL polypropylene syringes
  - 22 gauge blunt-tip dispensing needles
  - Drain tube assembly
- Inlet filter

Physical data

Substance	FW (/mol)	Quantity	MP (°C)	BP	Density (g/mL)
Acetic acid (anhydr.)	60.05	25 mL	118		1.049
Isopentyl acetate	130.1	product		142	0.876
Isopentyl alcohol	88.15	20 mL		130	0.809
Conc. H_2SO_4	98.08	5 mL			1.841
5% $NaHCO_3$	84.01	250 mL			1.0018
Sat. NaCl		10 ml			
Na_2SO_4 (anhydr.)	142.04				

Reaction

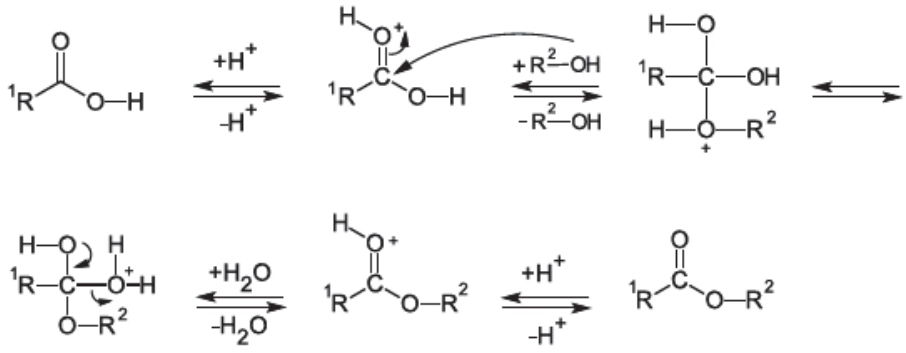


Acetic Acid

Isopentyl alcohol
(2-methylbutanol)

Isopentyl acetate
(2-methylbutyl acetate)

Mechanism



Safety Precautions

NOTICE Be sure that all persons operating this system read the site and safety manual first.

Experimental Procedure

1. To a 100 mL round bottom flask add 25 mL (0.420 mol) glacial acetic acid followed by 20 mL (0.185 mL) isopentyl alcohol (3-methyl-1-butanol).

   Swirl the flask to mix the layers.

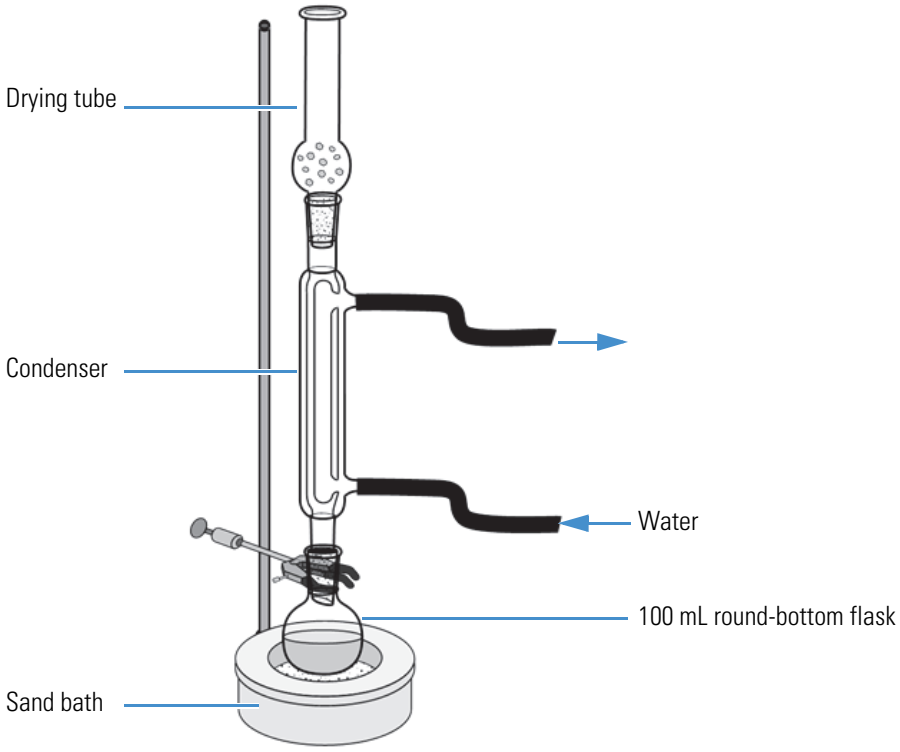
2. To the solution add (carefully, gloves) 5 mL concentrated sulfuric acid.

   Swirl the flask as sulfuric acid is added (heat generated).

3. Add several boiling chips to the flask, and then place a reflux condenser with lightly greased joints on the flask as shown in Figure 1.

Figure 1

Reflux apparatus with exclusion of moisture



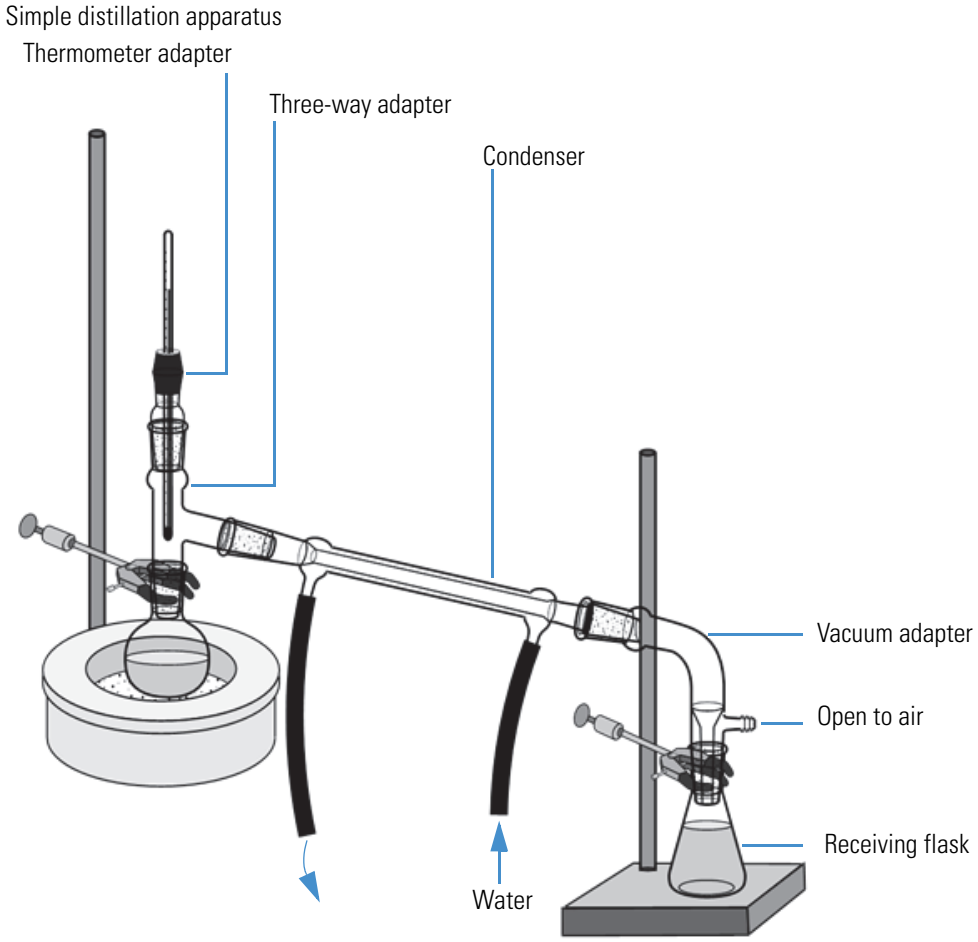
4. Bring the solution to boil with a sand bath, electric mantel or flame and reflux for 1 hour.
5. After reflux is completed, allow the solution to cool to room temperature.

   Transfer the entire solution to a separatory funnel and add 50 mL distilled water. Swirl the solution, allow the layers to separate, and remove the lower aqueous layer.
6. Add another 25 mL portion of distilled water, shake the flask, and separate and remove the lower aqueous layer.
7. Extract the organic layer with three 25 mL portions of 5% aqueous sodium bicarbonate solution to remove excess acetic acid.

   Note Be careful, as carbon dioxide is given off during the extraction.
8. Test the last extract and if the aqueous phase is not basic (pH paper), extract the organic layer with two more 25 mL portions of sodium bicarbonate solution ($NaHCO_3$).
9. After removal of the acetic acid, wash the organic layer with two 5 mL portions of saturated salt solution.
10. Transfer the organic layer to a 50 mL Erlenmeyer flask and dry over granular anhydrous sodium sulfate (Na_2SO_4) or magnesium sulfate ($MgSO_4$).

11. After drying (the liquid should be clear), decant the organic layer into a 50 mL round bottom flask.
12. Assemble a simple distillation apparatus, as shown in Figure 2.

Figure 2



13. Add several boiling chips and distill, using a sand bath, electric mantel or flame.
14. Cool the receiver flask in an ice bath.
15. Collect the fraction that distills between 135 °C and 143 °C.

    The clear colorless product has an intense odor of bananas; it should be obtained in 80 to 90% yield.

Analysis

Required:
Acquire ^{1}H NMR spectra of:

- Acetic acid (reactant)
- 3-Methyl butanol (reactant)
- 3-Methylbutyl acetate (distilled product)

Optional:

Acquire ^{1}H NMR spectra of:

- Initial reaction mixture (prior to reflux)
- Reaction mixture after reflux
- After H_2O wash and $NaHCO_3$ wash
- After $NaHCO_3$ wash, salt extraction and drying

NMR Sample preparation

Using a new, disposable 1mL polypropylene syringe fitted with a 1½ inch 22 gauge blunt-tip needle draw about 0.5 mL of glacial acetic acid and transfer it to a 0.5 or 1 dram vial.

If available, to this sample, using a new, disposable 1mL polypropylene syringe fitted with a 1½ inch 22 gauge blunt-tip needle, rapidly draw out and add 6 to 10 droplets of tetramethylsilane (TMS).

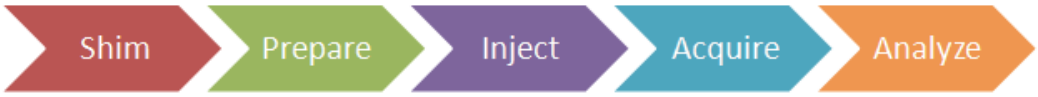
Note TMS will begin to boil immediately after insertion of the syringe needle. Thus, sample transfer must be rapid.

Repeat this procedure for preparing samples of 3-methylbutanol and 3-methylbutyl acetate for NMR analysis.

Note Some of the optional samples are aqueous and therefore TMS should not be added.

Instrumental Procedure

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



Shim

Ensure the NMR spectrometer is shimmed and ready to accept samples.

Presample preparation

1. Displace the shim fluid from the picoSpin capillary cartridge with air.
2. Flush the cartridge with 0.1 mL of chloroform, and then displace the solvent with an air push.
3. Set up the onePulse script according to parameters listed in the Pulse Script table.

NMR Sample preparation

Using a new, disposable 1mL polypropylene syringe fitted with a 1½ inch 22 gauge blunt-tip needle draw about 0.5 mL of glacial acetic acid and transfer it to a 0.5 or 1 dram vial.

If available, to this sample, using a new, disposable 1mL polypropylene syringe fitted with a 1½ inch 22 gauge blunt-tip needle, rapidly draw out and add 6-10 droplets of tetramethylsilane (TMS).

Note TMS will begin to boil immediately after insertion of the syringe needle. Thus, sample transfer must be rapid.

Repeat this procedure for preparing samples of 3-methylbutanol and 3-methylbutyl acetate for NMR analysis.

Note Some of the optional samples are aqueous and therefore TMS should not be added.

Injection

1. Using a 1 mL disposable polypropylene syringe fitted with a 1½ inch long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of sample.
2. Inject about half the sample.

   Ensure all air bubbles have been displaced from the cartridge by examining the drain tube.
3. Seal both the inlet and outlet ports with PEEK plugs.

Acquire

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

Pulse Script: onePulse

Parameter	Value
tx frequency (**tx**)	proton Larmor frequency (MHz)
scans (**ns**)	16 or 25
pulse length (**pl**)	Instrument specific 90° pulse length
acquisition time (**aq**)	750 ms
T1 recycle delay (**d1**)	10 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	-200 Hz
max freq. to plot	+1000 Hz
zero filling (**zf**)	8192
align-avg. data	✓
live plot	✓
JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default **pfa** values

Processing

Download the experimental JCAMP spectra files and open them by importing 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

Function	Value
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)	-

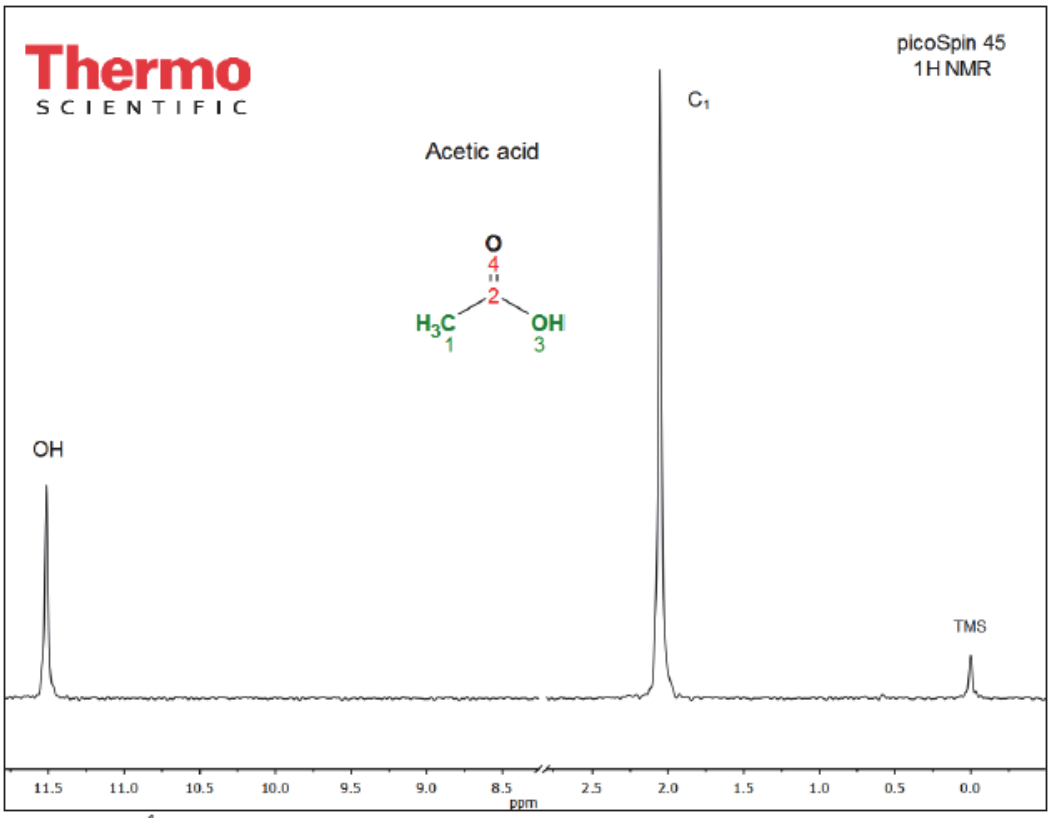
1. Import each data file into the same workspace in Mnova.
2. Manually apply Ph0 phase correction to each spectrum.
3. Manually shift reference each spectrum using Mnova's TMS tool.
4. Assign the TMS signal (0 ppm) or $CHCl_3$ signal (7.24 ppm), whichever is present.
5. Identify and assign each signal in the spectra.
6. Save the Mnova document, print each spectrum and paste into your lab notebook.

Results

Acetic acid (Figure 3) contains a carboxylic acid. Proton (^1H) 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 11and 12 ppm. Acidic protons also experience intramolecular hydrogen bonding and exchange rapidly; 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.

Figure 3

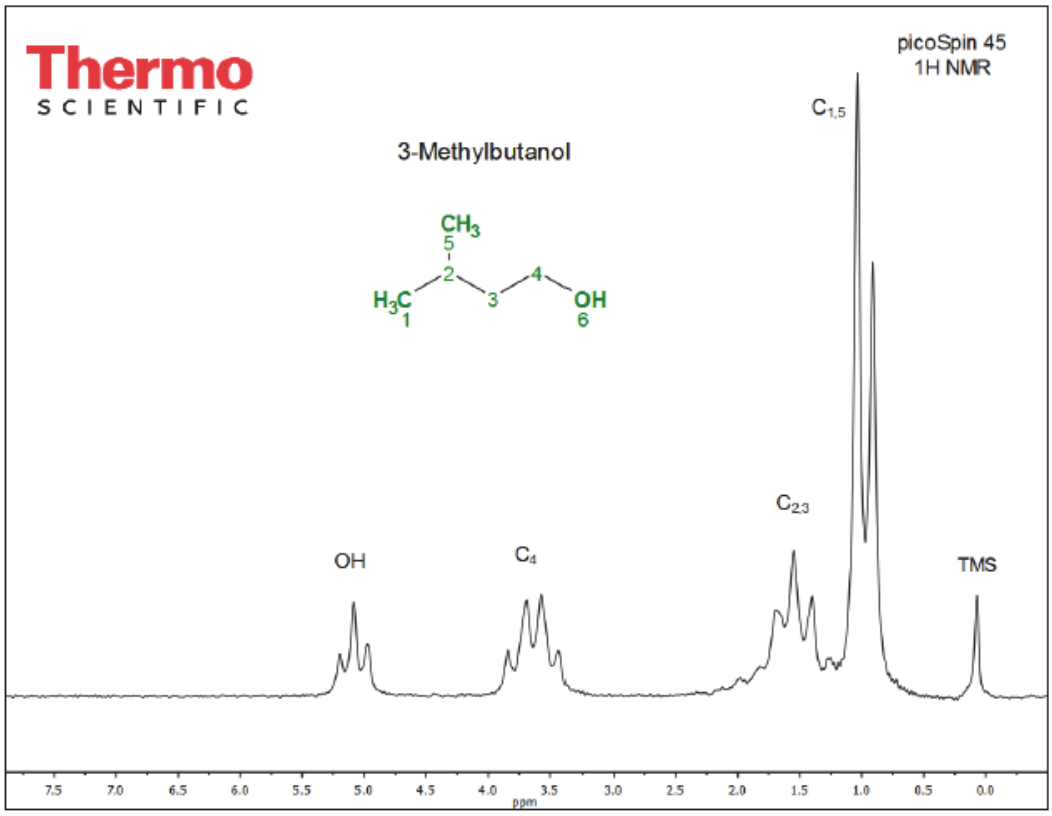
Full ^{1}H NMR (45 MHz) spectrum of acetic acid (anhdyr.; neat)



A characteristic feature of the ^{1}H NMR spectrum of 3-methylbutanol (Figure 4) is the presence of a coupled alcohol triplet appearing near 5 ppm. One reason a triplet structure is observed is that alcohol protons undergo dynamic exchange at various rates, fast exchange leads to hydroxyl protons that do not couple with neighboring protons, resulting in a singlet. However, slow exchange allows time for coupling, such as that observed in 3-methylbutanol where the hydroxyl group proton couples with two methylene protons on the neighboring C_4 carbon, resulting in a triplet. Steric hindrance and intramolecular bonding can also interfere with dynamic exchange, allowing for coupling to adjacent protons. The C_4 protons (3.5 ppm) in turn couples with the alcohol and two methylene protons at position C_3, generating a quartet signal instead of a triplet.

Figure 4

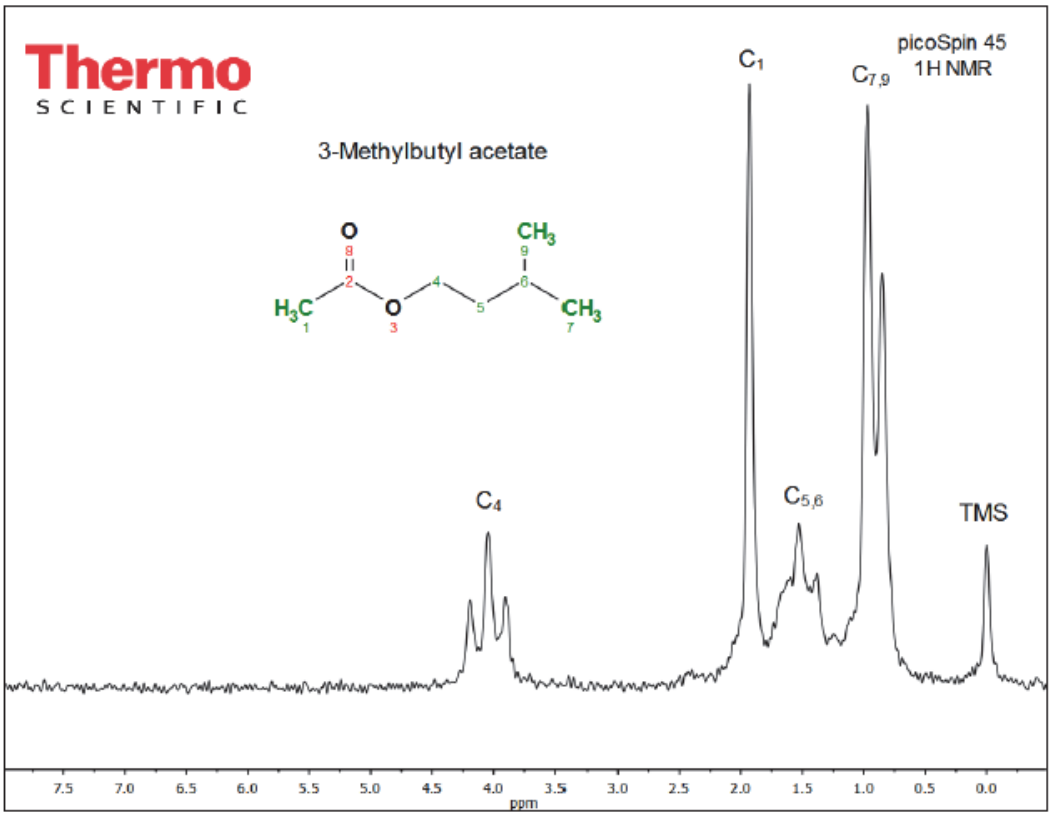
Full ^{1}H NMR (45 MHz) of 3-methylbutanol (neat)



As the reaction proceeds and 3-methylbutyl acetate is produced (Figure 5), two distinctive spectral features will appear its NMR spectrum. The alcohol signal of 3-methylbutanol will disappear and the C_4 proton quartet will change into a triplet due to the loss of an adjacent alcohol proton; it will also shift downfield by 0.5 ppm, from 3.5 ppm to 4.0 ppm. The downfield shift of C_4 protons arises from increased deshielding of these protons as the adjacent alcohol group is transformed into the more electron-withdrawing ester functional group. Furthermore, evidence of the formation of product is seen in the presence of a singlet group appearing at just below 2 ppm; this signal belongs to the methyl ester group (C_1) of the carboxyl group (Figure 3 and Figure 5). The remaining signal groups due to protons within the isobutyl group ($C_{1\text{-}3,5}$ in 3-methylbutanol; $C_{5\text{-}7,9}$ in 3-methylbutyl acetate) are largely unchanged upon esterification of 3-methylbutanol.

Figure 5

Full ^{1}H NMR (45 MHz) of 3-methylbutanol (neat)



Comments

picoSpin 45 ^{1}H NMR proton spectra of neat acetic acid, 3-methylbutanol and 3-methylbutyl acetate are show in Figure 3 to Figure 5. Chemical shifts and related NMR data are available in Table 1 ^{1}H NMR Spectral Data. Chemical shifts are referenced relative to TMS. Spectra are acquired from neat samples of reactants and product, and aliquots drawn from reaction mixtures. With the picoSpin NMR spectrometer, it is not necessary to dilute samples prior to injection. However, 3-methylbutanol is somewhat viscous resulting in broaden signals, more so than 3-methylbutyl acetate, and dilution to 50% in $CDCl_3$ can improve signal resolution. Using a labile deuterated NMR solvent will result in the hydroxyl (-OH) protons to exchange and its signal will diminish or disappear from the spectrum. Likewise, coupling to the methylene (C_4) proton will also be affected.

Table 1 ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
3	Acetic Acid	TMS	0	12 H	Singlet
		HO-C(=O)CH_3	2.05	3 H	Singlet
		HO-C(=O)CH_3	11.51	1 H	Singlet
4	3-Methylbutanol	TMS	0		Singlet
		-CH-$(CH_3)_2$	0.90	6 H	Doublet
		-CH_2-CH_2-CH-	1.47	2 H	Triplet
		-CH-$(CH_3)_2$	1.47	1 H	Multiplet
		-CH2-CH-(CH3)2	3.56	2 H	Quartet
		HO-CH_2CH_2	4.99	1 H	Triplet
5	3-Methylbutanol acetate	TMS	0	12 H	Singlet
		-CH-$(CH_3)_2$	0.91	6 H	Doublet
		-CH_2-CH_2-CH-	1.53	2 H	Triplet
		-CH_2-CH$(CH_3)_2$		1 H	Multiplet
		CH_3COO-	1.93	3 H	Singlet
		O=CO-CH_2-CH_2	4.05	2 H	Triplet

Presented in Figures 6 - 8 are stacked ^{1}H NMR spectra acquired from neat reactants, isolated product, and spectra acquired from the reaction mixture during various stages of the experiment. These spectra are instructive in that they demonstrate the need for proper 'work up' of the reaction mixture prior to product distillation and isolation. Moreover, it is easy to visualize the changes in ^{1}H NMR spectra as reactants are converted to products.

Figure 6 compares reactants and products, while Figure 7 includes the initial reaction mixture prior to addition of the acid catalyst (H_2SO_4). We see a hydroxyl signal appearing near 9.3 ppm, the apparent change of the methylene quartet to a triplet (~3.5 ppm), the appearance of a second methyl ester 'singlet' below 2.0 ppm and the broadening of all signals. The chemicals shift of the –OH group reflects the rapid exchange between the carboxylic acid proton and the alcohol which coalesce into one signal. Its position is dictated by relative mole fractions of each component (acid and alcohol); that is, its chemical shift is linearly dependent on the mole fraction of the two labile protons under exchange. This is a well-known phenomenon to occur with mixtures of alcohols.

Figure 6

Full, stacked and labeled ^{1}H NMR (45 MHz) spectra of acetic acid and 3-methylbutanol (reactants), and 3-methylbutyl acetate (product)

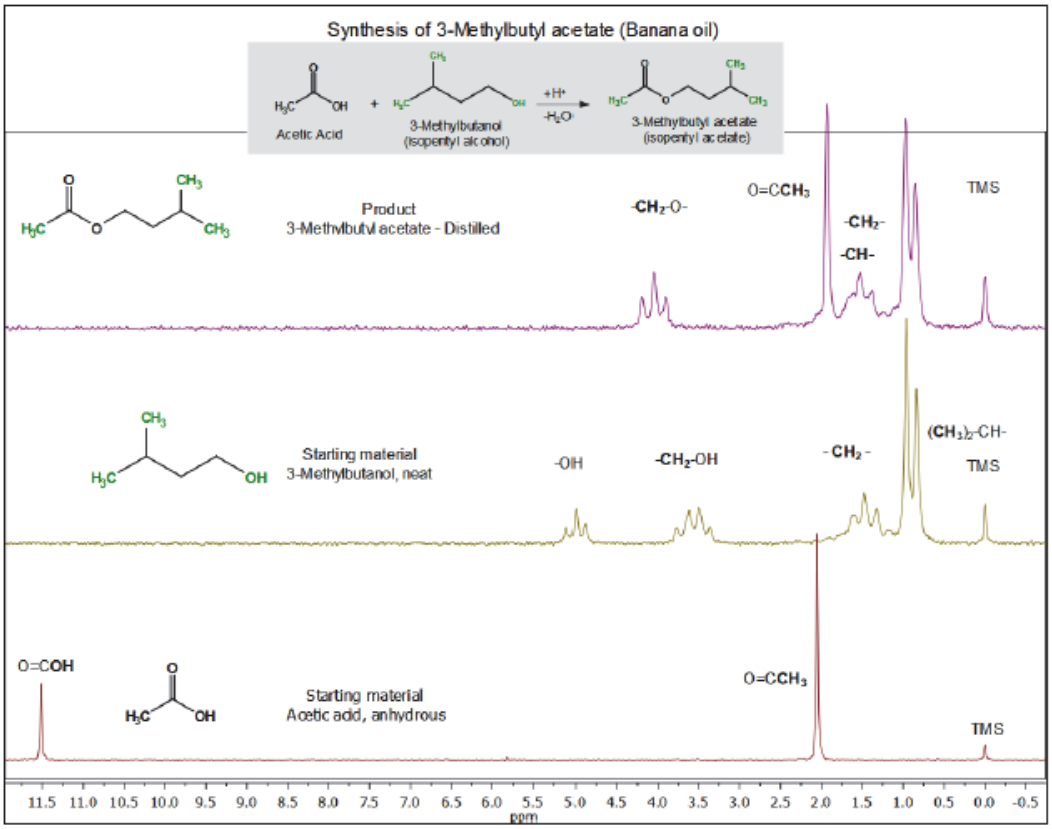
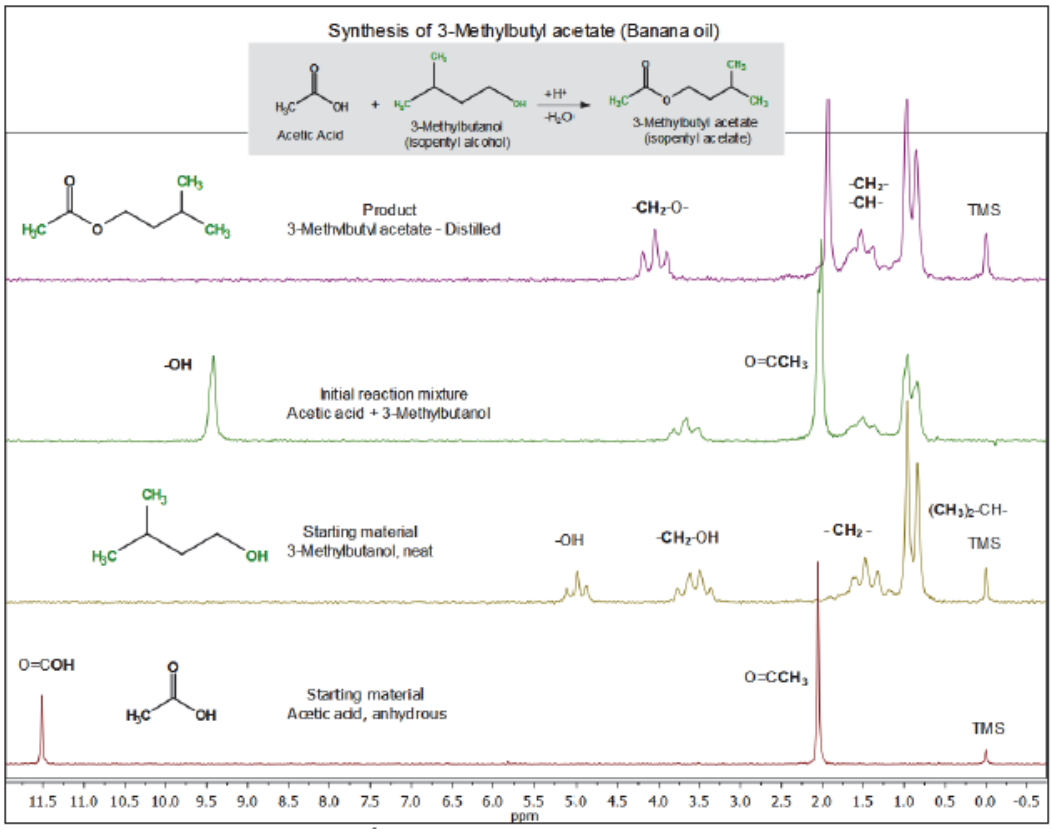


Figure 7

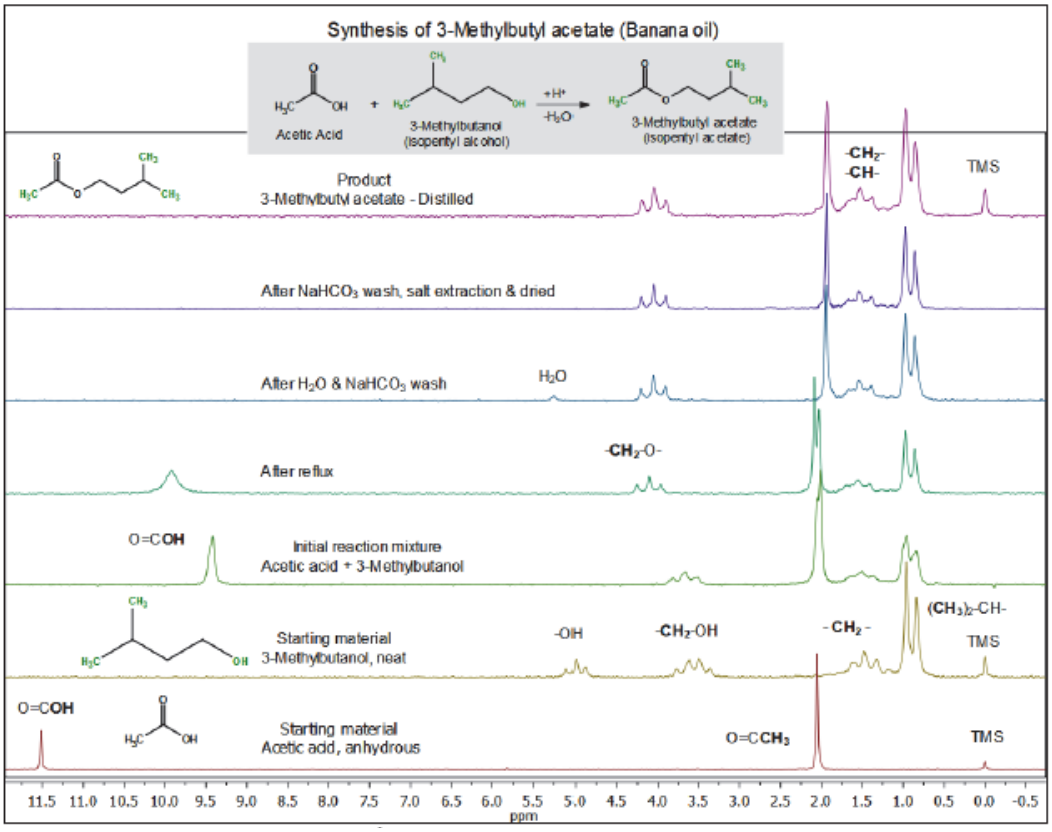
FulI, stacked and labeled ^{1}H NMR (45 MHz) spectra of acetic acid and 3-methylbutanol (reactants), 3-methylbutyl acetate (product), and the initial reaction mixture prior to reflux.



Looking at Figure 8 we see in the 'after reflux' spectrum (spectrum 4th from the bottom) distinct and resolved singlet resonances due to the each type of carboxyl methyl group; one at ~2.05 ppm from acetic acid and a second one at ~1.93 ppm arising from the methyl ester product. The methylene ester (-CH_2-O-) appears shifted downfield to ~4.0 ppm and is a clear triplet structure. In addition, due to the presence of mineral acid (H_2SO_4) the coalesced hydroxyl/carboxylic acid signal, appearing at ~9.3 ppm in the initial reaction mixture, is further broaden and is now centered on ~9.7 ppm. The additional downfield shift arises from an increase of H+ ions to the mixture.

Figure 8

Full, stacked and labeled ^{1}H NMR (45 MHz) spectra of acetic acid and 3-methylbutanol (reactants), 3-methylbutyl acetate (product), as well as spectra acquired after different times during the experiment.



After a water wash and neutralization with sodium bicarbonate ($NaHCO_3$), the merged labile hydroxyl/carboxylic acid proton signal (9.7 ppm) and the carboxylic acid signal (2.05 ppm) disappear (spectrum 3rd from top). The methylene ester ($-CH_2-O-$) shows better resolution; also, a residual water signal is present. An additional $NaHCO_3$ wash, followed by salt extraction and drying of Na_2SO_4 (or $MgSO_4$) cleans up the product spectrum prior to distillation (spectrum 2nd from top). The top two spectra in Figure 8 establish that even prior to distillation, the product 3-methylbutyl acetate is well isolated, and the 'before and after' distillation spectra appear nearly identical.

Own Observations

Ordering Information

To reorder this lesson plan from the *picoSpin NMR Spectroscopy: Example Lesson Plans* set, please refer to document order number: "**LP52589_E 05/14M** picoSpin Lesson Plan #2-Fisher Esterification Reaction Synthesis"

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E-mail: us.techsupport.analyze@thermofisher.com

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Hydrolysis of Acetic Anhydride with Heavy Water (D_2O): picoSpin 45 or picoSpin 80

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:

- reactants and products must be soluble throughout the course of reaction
- signals undergoing change must be resolvable
- 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, $1/t$, 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.

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 or Thermo Scientific™ picoSpin™ 80 ^{1}H NMR spectrometers. 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.

Literature

Binder, D. A.; Ellason, R.; Axtell, D. D., Kinetic hydrogen isotope effect, *J. Chem. Educ.,* **1986***, 63*, 536.

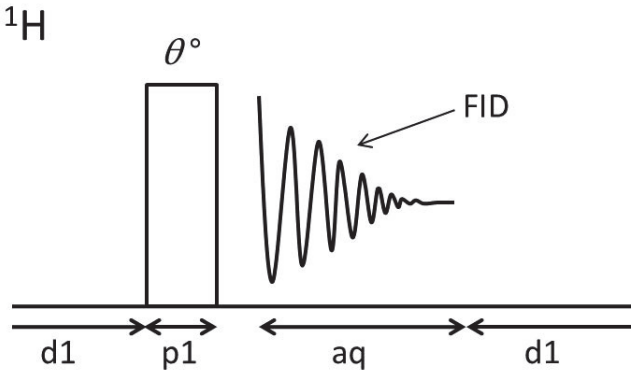
Gold, V. The Hydrolysis of Acetic Anhydride, *Trans. Faraday Soc.,* **1948**, *44*, 506-518.

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

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: Structure and Mechanisms*, 2nd ed., Plenum Press, pp 190-194.

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 tim (ms)
ns: # of scans (individual FIDs)

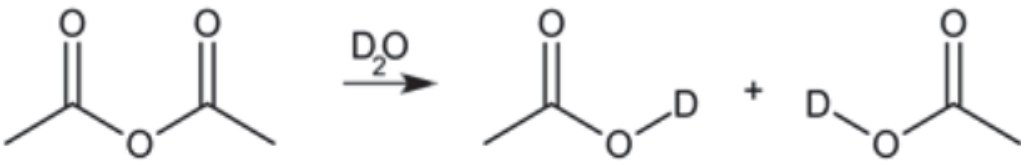
Procedure and Acquisition

Time requirements: 45 minutes
Sample: 2% (v/v) acetic anhydride in deuterium oxide (D_2O)
Difficulty: Easy

Equipment/material:

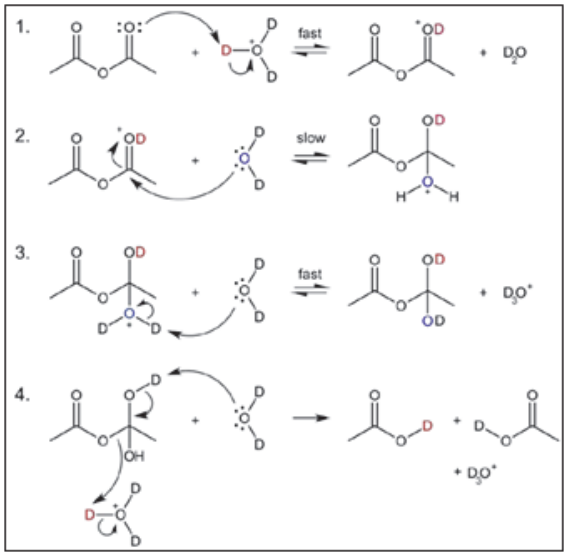
- picoSpin 45 or picoSpin 80
- Acetic anhydride ($C_4H_6O_3$)
- Deuterium oxide
- 1 mL vial with PTFE cap liner
- 1 mL polypropylene syringes
- 22 gauge blunt-tip dispensing needles
- Mnova™ NMR Processing Suite
- picoSpin accessory kit
  - Drain tube assembly
  - Syringe port adapter
  - Port plug

Reaction



Acetic anhydride Acetic acid-d

Mechanism

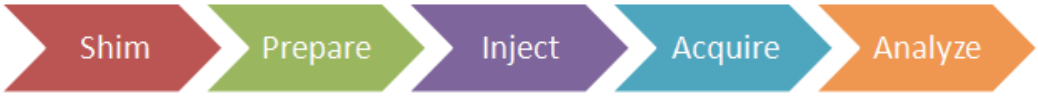


Physical data

Substance	FW (g/mol)	Quantity	MP (°C)	BP	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.

Prereaction Preparation

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

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

Reaction preparation

1. To 0.5 mL of D_2O in a 2 mL vial, add 0.01 mL of acetic anhydride.
2. 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

1. Using a 1 mL disposable polypropylene syringe fitted with a 1½ inch long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of the reaction mixture.
2. Inject about half the sample.

   Ensure all air bubbles have been displaced for the cartridge by examining the drain tube.
3. Seal both the inlet and outlet ports with PEEK plugs.

Acquire

1. Execute the onePulse script according to the values in the table of parameters provided.
2. 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 (**pl**)	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)

Parameter	Value
exp. filter (**LB**)	0 Hz
max plot points	400
max time to plot	250 ms
min freq. to plot	-400
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

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 $\rightarrow$ 16 k
Backward predict (BP)	From -2 $\rightarrow$ 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)	-

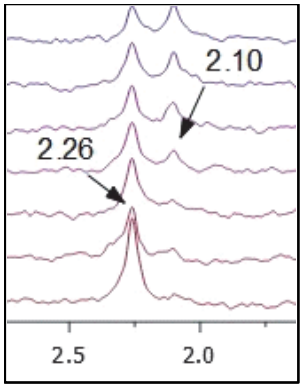
1. Import each data file into the same workspace in Mnova.

   There should be 30 NMR spectra displayed in the **Pages** view in Mnova.

2. Highlight all spectra and process them simultaneously, including Ph0 phase correction.

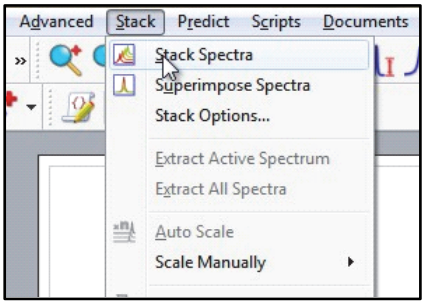
3. Manually shift reference each spectrum using Mnova's TMS tool by assigning a chemical values of 2.10 ppm for the right most signal, or 2.26 just to the left of this signal.

   It is important that the spectra be properly referenced.



4. 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).

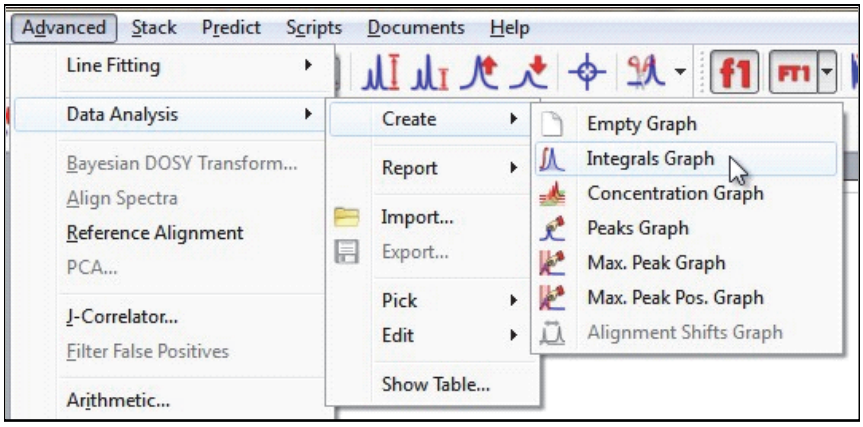
   A new stacked spectrum page will appear.



5. Zoom into the stacked spectra to display a chemical shift range from about -0.5 to 6.0 ppm.

6. Select '/Advanced/Data Analysis/Create/Integrals Graph'.

   An 'integration' icon will appear.



7. Highlight signal at 2.26 ppm and an integrated peak area vs. data array (time) time data plot will appear.
   a. Select the region containing the Ac_2O signal for integration and a small data plot will appear above the stacked spectra.
   b. 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**.
8. Re-phase the spectra and repeat the data analysis for the second peak at 2.26 ppm.
9. Highlight the signal at 2.10 ppm and an integrated peak area vs. data array (time) time data plot will appear.
   a. 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.
   b. Select the region containing the AcOD signal for integration and a small data plot will appear above the stacked spectra.
   c. 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.

Results

The predicted ^1H NMR spectrum for equi-molar quantities of the reactant acetic anhydride (Ac_2O) and product acetic acid-d (AcOD) is presented in Figure 1a and Figure 1b, for 45 MHz and 82 MHz respectively. The spectrum contains only two signal, 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 proton (^1H) 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 1a

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

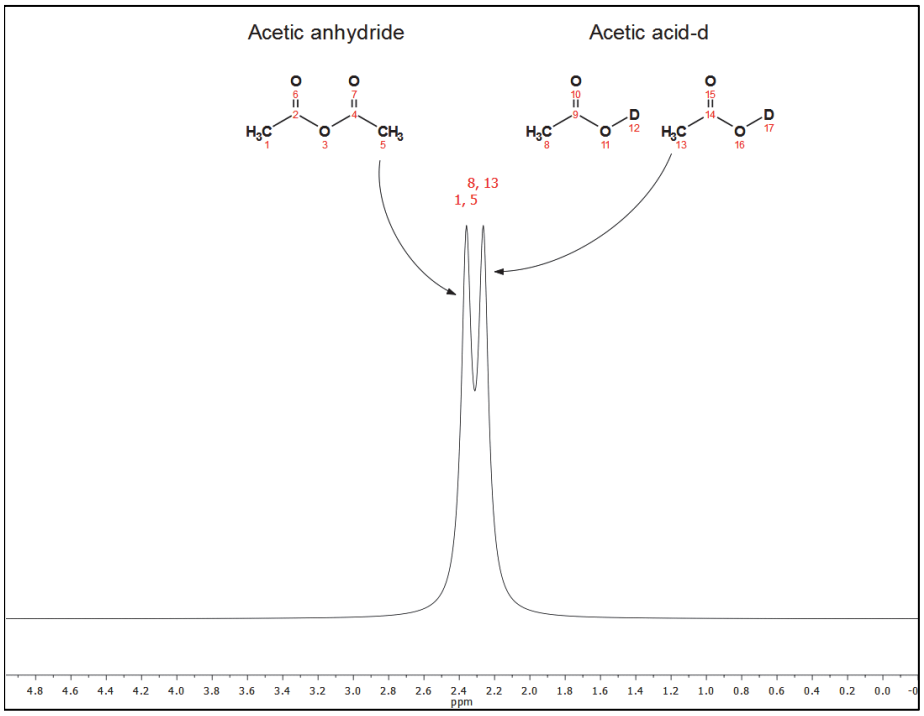
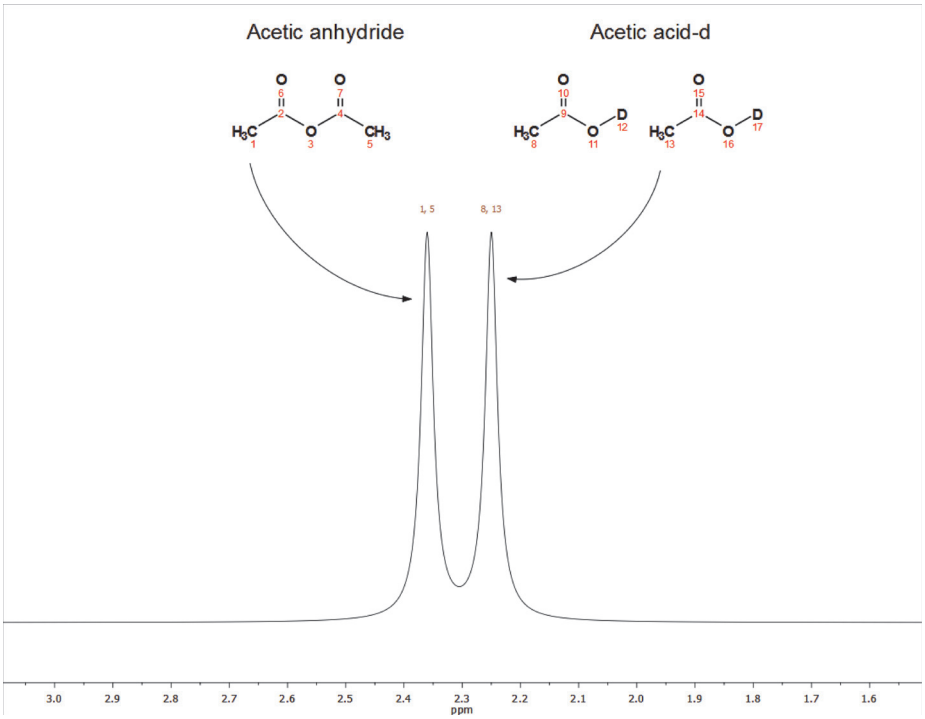


Figure 1b

Predicted ^{1}H NMR (82 MHz) spectrum of acetic anhydride and acetic acid-d



Acetic acid contains a carboxylic acid. Proton (^{1}H) 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 and 2 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 2a

Stacked ^1H NMR (45 MHz) spectrum plot of the reaction mixture acquired at 1minute intervals

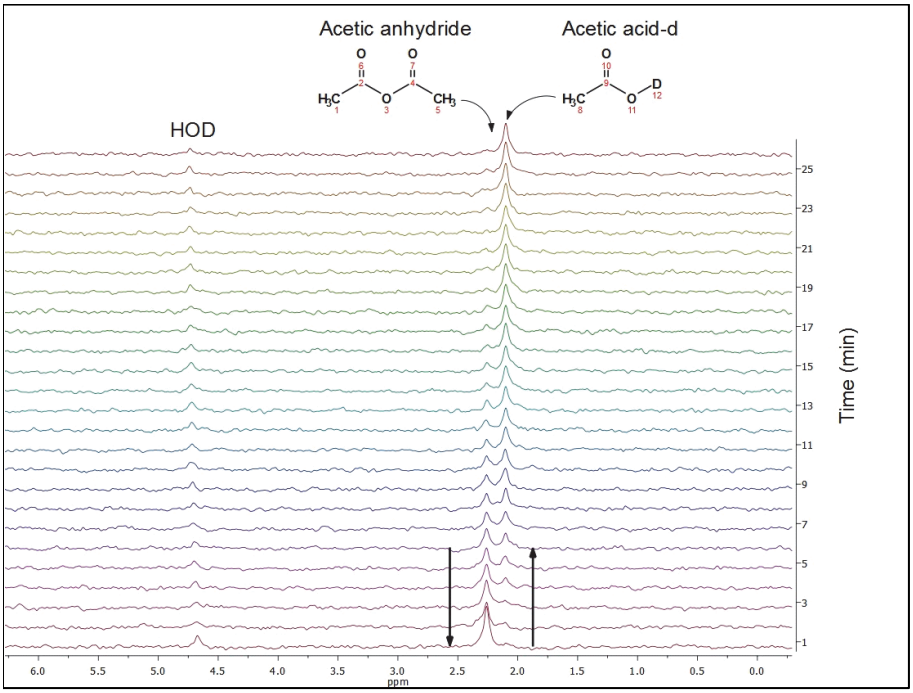
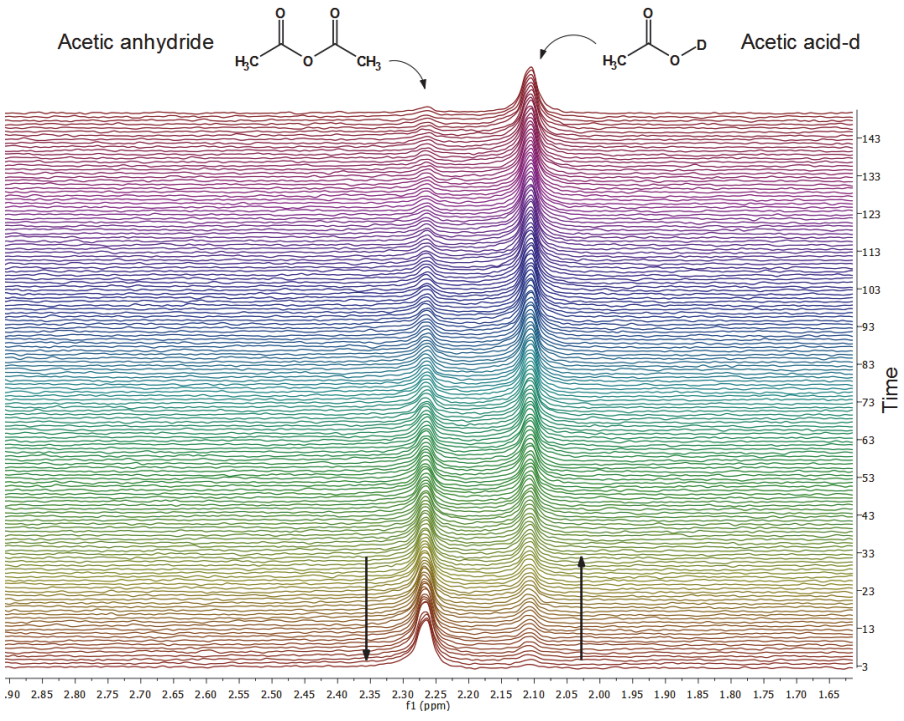


Figure 2b

Stacked ^1H NMR (82 MHz) spectrum plot of the reaction mixture acquired at 15 second intervals



Initially, at t = 0 min, the NMR spectrum of the reaction mixture (Figure 2a and Figure 2b) contains two signals, one at 4.6 ppm and is assigned to a residual water signal, HOD, 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 appearing at 2.10 ppm develops. The new signal is due to the product AcOD. At t ≈ 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. At t ≈ 30 min the reaction is nearly completed and the NMR spectrum is dominated by the product signal.

Figure 3a

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.

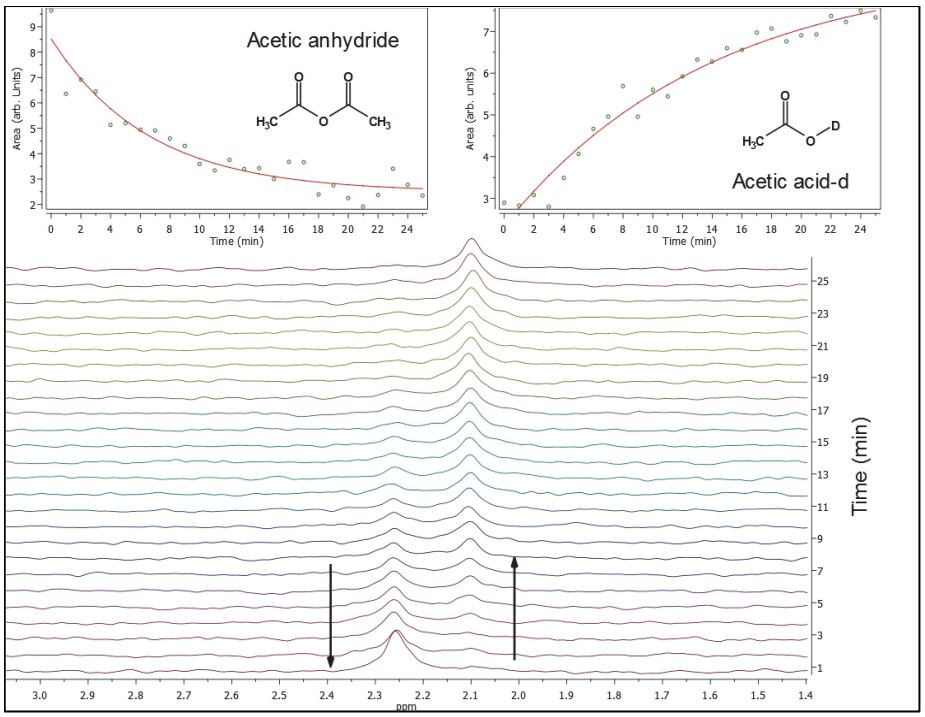
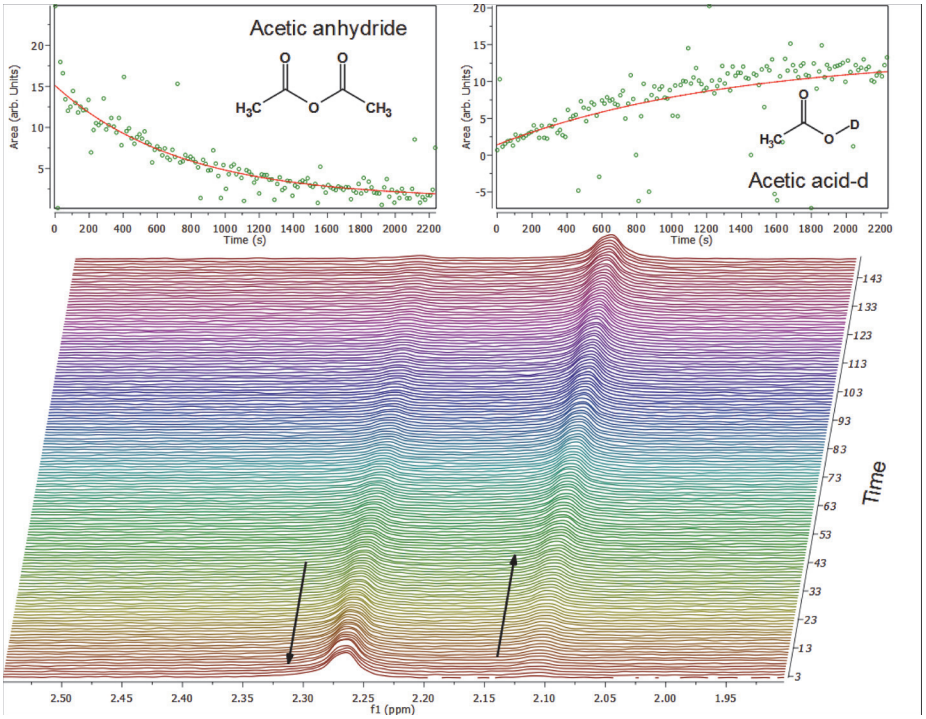


Figure 3b

Stacked ^1H NMR (82 MHz) spectrum plot of the reaction mixture acquired at 15 second intervals. Data plots displaying area plotted against time for the 2.26 ppm and 2.10 ppm



Integrating the peak areas of the reactant produces pseudo-first order rate curves as seen in Figure 3a and Figure 3b. 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.

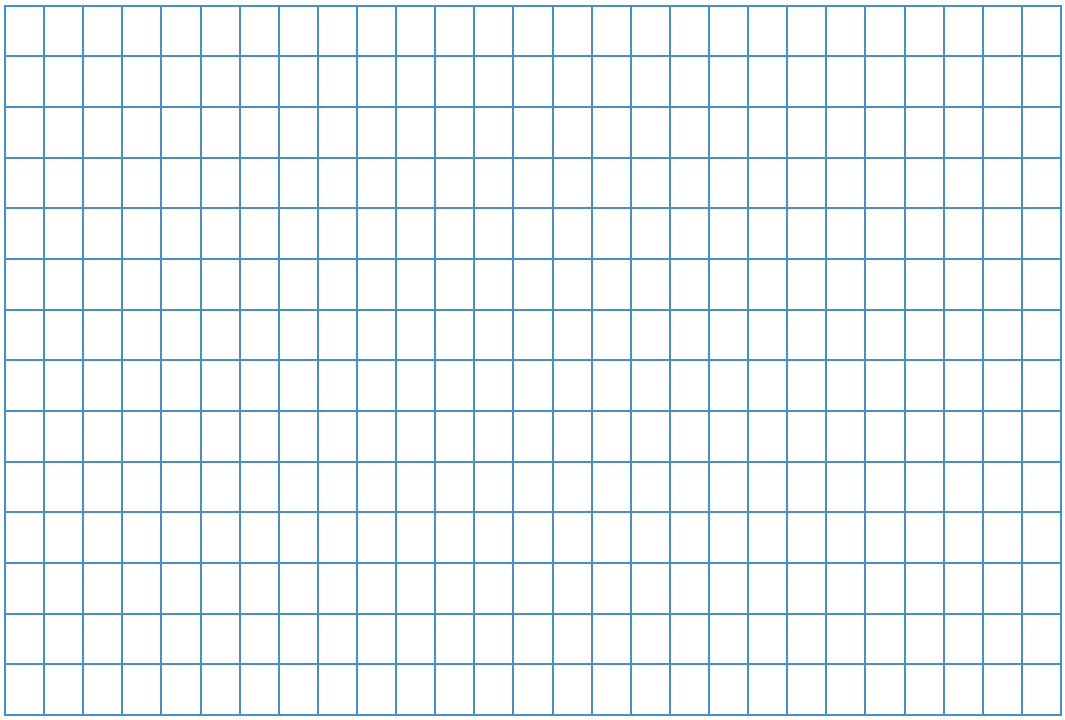
Comments

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. ^1H 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

Own Observations



Ordering Information

To reorder this lesson plan from the *picoSpin NMR Spectroscopy: Example Lesson Plans* set, please refer to document order number: "**LP52590_E 05/14M** picoSpin Lesson Plan #3-Hydrolysis of Acetic Anhydride"

For U.S. Technical Support, please contact:

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Madison WI 53711-4495 U.S.A.
Telephone: 1 800 532 4752
E-mail: us.techsupport.analyze@thermofisher.com

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Note Please have the instrument serial number available when you contact us.

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Friedel-Crafts Acylation of Ferrocene: picoSpin 45 and picoSpin 80

Introduction

The Friedel-Crafts reaction represents a very important and broad class of electrophilic aromatic substitution reactions. The acylation reaction utilizes a Lewis acid catalyst, such as BF_3 or $AlCl_3$, to produce an acyl cation that adds to the aromatic ring. Important reagents for acylation are acyl halides, carboxylic acids, anhydrides and ketenes. The alkyl group, R, in the acylating reagent can be an aryl or alkyl group. Acylation does not suffer from R-group rearrangement to a more stable carbocation species suffered by R groups Friedel-Crafts alkylation reactions, a major disadvantage, because electrophilic attack in acylation is via an acylium ion (an acyl cation, $RC{=}O^+$). With anhydrides, the mineral acid phosphoric acid (H_3PO_4) can be used as the Lewis acid catalyst, and acylation with nitriles (RCN; the *Hoesch reaction*) employs HCl and $ZnCl_2$.

Acylation requires an electron rich aromatic ring system and cannot contain any electron withdrawing substituents on the ring. Ferrocene (bis(η^5-cyclopentadienyl)iron; $Fe(C_5H_4COCH_3)_2$) is an organometallic compound containing iron (Fe) "sandwiched" between two cyclopentadienyl rings opposite the central metal atom. The cyclopentadienyl rings are aromatic according to Hückel rule, they are planar, cyclic, conjugated and satisfy the 4n+2 rule. Because of their high electron density, acylation of ferrocene is accomplished under milder conditions using a phosphoric acid as acid catalyst. The acyl group (RCO) is deactivating, stop the reaction cleanly after the addition of one group per aromatic ring. Thus, in this microscale Friedel-Crafts acylation reaction of ferrocene with acetic anhydride using a phosphoric acid as the Lewis acid catalyst, the major reaction is acetylferrocene ($[Fe(C_5H_4COCH_3)(C_5H_5)]$), with minor presence of diacetylferrocene ($Fe(C_5H_4COCH_3)_2$). The reaction product is isolated and purified by microscale flash column chromatography.

Column chromatography is one of many basic laboratory techniques taught in organic chemistry. It has widespread application in the organic synthetic lab because of its efficiency for separating and purifying components of a mixture. It can be applied to both liquid and solid samples, and multi-component mixtures. On a small scale, column chromatography is fast and cost effective. It is particularly useful for separating reaction mixtures containing reactants, products and byproducts.

Chromatography takes advantage of differences in polarity and binding strength components of a mixture have for column adsorbents. Adsorbents are high surface area stationary phase materials that bind solute molecules. A mobile phase solvent, or eluent, is used to desorb solute molecules, carrying them along the column to a receiving flask. As the solvent polarity is increased, polar molecules bound more strongly to the column begin to solubilize and are carried down the column in the mobile phase. Equilibrium is established between binding to the stationary phase and solubility in the mobile phase. As the solvent polarity increases, more tightly bound polar molecules, firmly held by the adsorbent, establish equilibrium with the eluting solvent and flow along the column. This process is analogous to thin layer chromatography (TLC), gas chromatography (GC) and high-performance liquid-phase chromatography (HPLC).

Purpose

The purpose of this experiment is to perform a microscale synthesis of acetylferrocene from ferrocene and acetic anhydride in an acid-catalyzed (85% H_3PO_4) Friedel-Crafts reaction. The crude reaction product is isolated by filtration and purified on a silica gel column using flash chromatography. Two fractions will be collected from the column separation of the mixture. Ferrocene (fraction 1) is eluted first using hexanes; it appears as a yellow band in the column and represents excess reagent. The product, acetylferrocene (fraction 2), is eluted next using a 50:50 mixture of hexanes and diethyl ether solution.

Solutions of pure ferrocene and acetylferrocene, crude isolated product and column purified reaction product will be prepared and analyzed using the Thermo Scientific™ picoSpin™ 45 and picoSpin™ 80 NMR spectrometers.

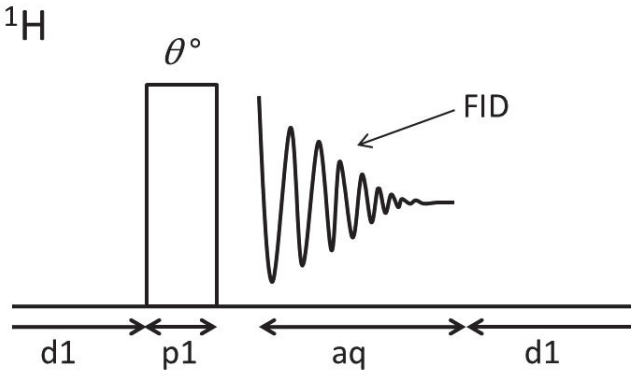
Literature

Adapted from Williamson, K. L.; Minard, R.; Masters, K. M. *Macroscale and Microscale Organic Experiments*, 5th ed., Houghton Mifflin Co., **2007**.

Bozak, R. E. Acetylation of Ferrocene *J. Chem. Ed*. **1966**, *43*, 73.

Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



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 tim (ms)
ns: # of scans (individual FIDs)

Procedures and Analysis

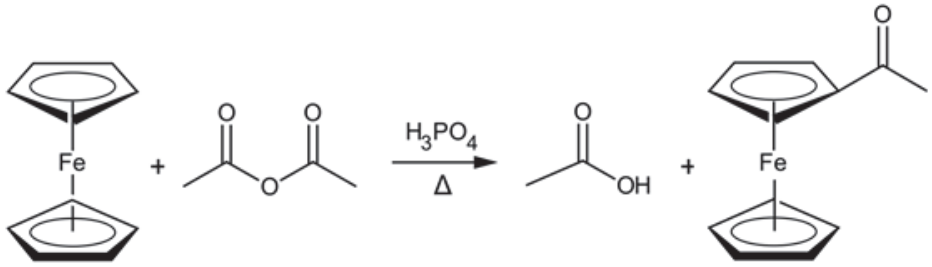
Time requirements: 3 to 3.5 hours
Difficulty: Moderate
Sample: Ferrocene, acetylferrocene

Equipment/materials:

- picoSpin 45 or picoSpin 80
- Ferrocene ($C_{10}H_{10}Fe$)
- Acetic anhydride ($C_4H_6O_3$)
- Phosphoric acid
- Hexanes
- Diethyl ether
- Dichloromethane
- NMR solvent: $CDCl_3$ w/1% TMS
- NMR solvent: Acetone-d_6 w/1% TMS
- Test tube (13 x 100mm)
- Silica gel (230-425 mesh), or alumina
- Tapered Collar neoprene filter adapter
- Several 7 mL vial with PTFE cap liner
- 1 mL polypropylene syringes
- Pipet bulb
- Polypropylene funnel
- Weighing paper/boat
- Cotton swab/ball
- Hirsch funnel
- Filter paper
- pH paper or litmus paper
- Ring stand, ring clamp, iron ring
- 25 mL vacuum flask
- Septum stopper
- Several 10, 25 and 50 mL beakers
- Mnova NMR Processing Suite
- picoSpin accessory kit:
  - Port plug

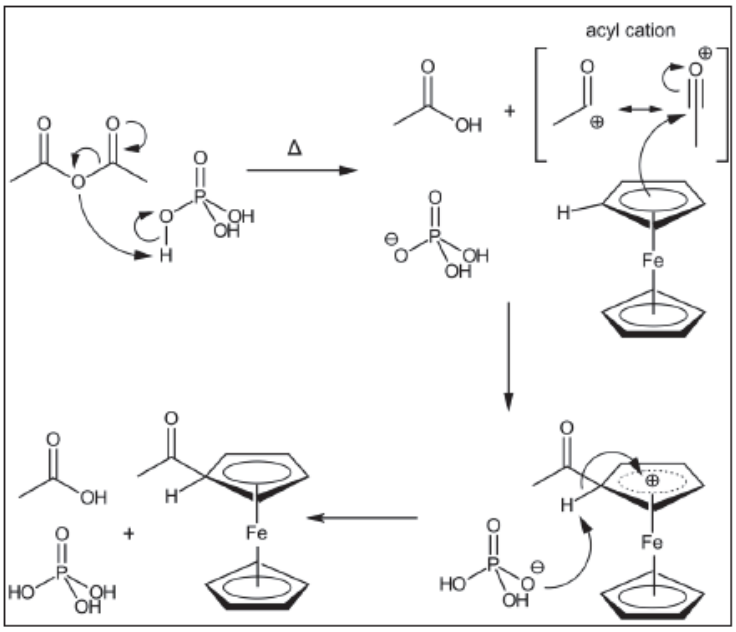
- 22 gauge blunt-tip dispensing needles
- Pasteur pipet
- Syringe port adapter
- Drain tube assembly

Reaction



Ferrocene Acetic anhydride Acetic acid Acetylferrocene

Mechanism



Physical data

Substance	FW (g/mol)	Quantity	MP (°C)	BP	Density (g/mL)
Ferrocene	186.04	180 mg	172.5		1.107
Acetic anhydride	102.09	10 µL	73.1	139.8	1.08
Phosphoric acid (H_3PO_4), 85%	98	150 mg			1.88
Acetyl ferrocene	228.07		81-8		
Hexanes	86.18	10-15 mL	-95	68-69	0.655

Substance	FW (g/mol)	Quantity	MP (°C)	BP	Density (g/mL)
Diethyl ether	74.12	10-15 mL	-116.3	34.6	0.7134
Dichloromethane	84.93	2 mL	-96.7	41	1.33
Chloroform-d ($CDCl_3$) w/1%TMS	120.384	1 mL	-64	61	1.50
Chloroform[a]	119.38	1 mL	-82.3	61.2	1.48
Acetone	59.08	1 mL	-95	56	0.791
Acetone-d_6 (Ac-d_6) w/1%TMS[a]	64.12	1 mL	-94	56	0.872

[a] Optional NMR solvents

Safety Precautions

NOTICE Be sure that all persons operating this system read the site and safety manual first.

Experimental Procedure

Reaction procedure

1. Add 184 mg of ferrocene to a 13 x 100 mm reaction test tube.
2. Add 0.70 mL (0.76 mg) of acetic anhydride
3. Add 0.2 mL (340 mg) of 85% phosphoric acid.
4. Cap the tube with a septum bearing an empty syringe needle.
5. Warm the reaction tube on a steam bath or in a beaker of hot water.
6. Agitate the mixture to dissolve the ferrocene.
7. Once dissolved, heat the mixture an additional 10 min and then cool the tube thoroughly in an ice bath.
8. Dropwise, carefully add 1 mL of ice water - mix thoroughly.
9. Dropwise, carefully add 3 M aqueous sodium hydroxide solution until the mixture is neutral (~3 mL) - use pH paper; avoid an excess of base.
10. Collect the product on a Hirsch funnel (or by gravity filtration).
11. Wash the product thoroughly with water.
12. Press it dry between sheets of filter paper.

13. Save a sample for melting point and TLC analyses.
14. Purify the remainder by column chromatography.

Prior to packing the column, label and tare (weigh) several 7 mL vials and two 25 mL flasks for receiving eluting fractions.

Preparing Samples

Several samples will be prepared for analysis. These solutions can be prepared in chloroform ($CHCl_3$), chloroform-d ($CDCl_3$) or acetone-d6 (Ac-d_6). If $CHCl_3$ is used then its proton NMR signal, at 7.24 ppm, can be used to shift reference the spectrum, otherwise the TMS signal (0 ppm) in $CDCl_3$ or Ac-d_6 is used. The sample preparation guide and spectra presented are for $CDCl_3$ solutions.

1. Prepare about 10 mL of a 50:50 mixture of hexanes and diethyl ether in a flask.
2. Stopper the flask to prevent diethyl ether from evaporating.

   Sample 1: To a tared, labeled vial measure about 30 mg of ferrocene and dissolve in 200 µL of $CDCl_3$. Record the sample weight. Cap and save for NMR analysis.

   Sample 2: To a tared, labeled vial measure about 30 mg of acetylferrocene (Caution: toxic) and dissolve in 200 µL of $CDCl_3$. Record the sample weight. Cap and save for NMR analysis.

   Sample 3: To a tared, labeled vial measure about 20 to 30 mg of dried crude reaction product and dissolve in 200 µL of $CDCl_3$. Record the sample weight. Cap and save for NMR analysis.

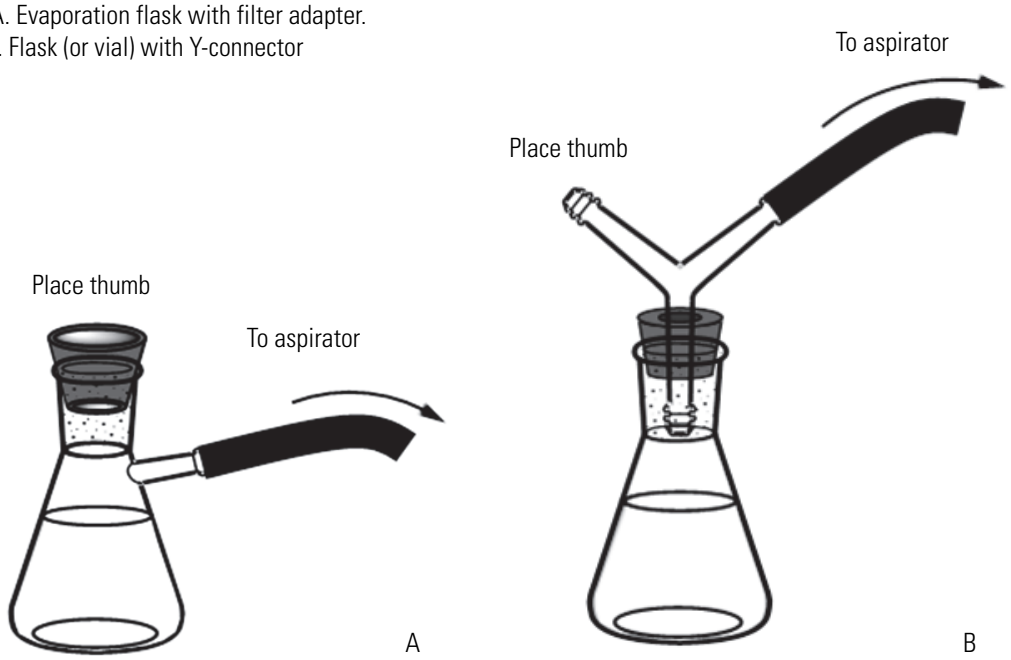
   Chromatography Sample 4: To a tared, labeled vial measure the balance of the crude reaction product. Record the sample weight. Dissolve in a minimum amount of dichloromethane. Once dissolved, add about 300 mg of silica gel (pre-weighed) and thoroughly mix the solution. Evaporate off the solvent in a hot water bath being careful not to cause bumping. (Reminder: dichloromethane boils at 41°C.). This sample is used for chromatography.

Chromatography fractions

Prior to preparing these samples, the eluting solvent must be evaporated off. A quick way to evaporate the solvent is under vacuum. Use a filter flask connected to a faucet aspirator and fitted with tapered collar neoprene (or 1-hole rubber stopper) as shown in Figure 1. Using your thumb control the vacuum pressure. Under reduced pressure, the boiling point of the eluting solvents lower to where the warmth of your hand is sufficient to cause boiling. Alternatively, in the absence of 25 mL vacuum flasks a similar apparatus can be made with a regular flask (or vial) using a 1-hole stopper and a Y-connector.

Figure 1

A. Evaporation flask with filter adapter.
B. Flask (or vial) with Y-connector



Fraction 1: Collect fraction 1 in a tared, labeled 25 mL vacuum flask, 25 mL flask or 7 mL vial. Dry, weigh and record the amount of sample recovered. Add 200 µL of $CDCl_3$. Cap and save for NMR analysis.

Note There should be very little ferrocene remaining. If less than 15 mg of material is available then only prepare a solution for Fraction 2.

Fraction 2: Collect fraction 2 in a tared, labeled 25 mL vacuum flask, 25 mL flask or 7 mL vial. Dry, weigh and record the amount of sample recovered. Add 200 µL of $CDCl_3$. Cap and save for NMR analysis.

Packing the Column

Whether using a microscale or macroscale technique, it is important, and critical to the success of column chromatography, to pack the column uniformly such that it is free of air pockets and gaps. Two methods for column packing are used: dry packing and slurry packing. In dry packing, the adsorbent, silica gel or alumina, is added directly to the column and 'tapped' into place to remove cracks, gaps and air pockets, then the first eluting solvent is added to wet the column. With slurry packing, a slurry mixture of adsorbent and first eluting solvent is prepared and then poured into the column. The preferred method is slurry packing as it has a higher chance of minimizing gaps and air bubbles, but can be a difficult technique to perfect since adsorbent must stay suspended during transfer to the column.

Dry Packing Method

For microscale columns using a Pasteur pipet, the dry packing method is preferred.

1. Remove the cotton end of a cotton swab, or an equivalent pea-sized cotton ball, and plug a Pasteur pipet.

   Use a wooden stick or stir rod to tamp it into place. Do not pack it too tightly.

2. Measure about 5 mL of adsorbent using a 10 mL beaker.

3. Add dry adsorbent to a height of about 5-6 cm. Leave ~3 cm at the top for the pre-adsorbed.

   Folded weighing paper is helpful in transferring adsorbent to the column.

4. Pack the column by alternately by gently tapping the side of the column with a stir rod or spatula, and tamping it on the bench top.

   Add more column pack material as need.

5. Once chromatography sample 4 is prepared, pre-elute the column with hexanes (the first eluent).

   Do not let the column dry out; allow a small volume of eluent to remain above the column pack.

6. Use a disposable syringe or pipet to add additional solvent.

Slurry Packing Method

Slurry packing a Pasteur pipet column is difficult because the pipet opening is small and the slurry needs to be introduced rapidly. This technique is better suited to macroscale chromatographic column preparation, such as with a burette.

1. Fill a 25 mL beaker to about the 5 mL line with adsorbent.

2. Add hexanes to initially wet the adsorbent, then while swirling add additional hexanes to produce a slurry.

   Note Increase the quantity of adsorbent and solvent accordingly to accommodate larger columns. With a burette, close the stopcock, and add solvent about a third of the way up the column.

3. When ready, swirl the mixture to produce a suspension, simultaneously removing air bubbles, and then quickly pour the slurry into the column.

4. Fill to a height of about 5 to 6 cm. Leave ~3 cm at the top for the pre-adsorbed.

5. Tap the side of the column to cause the adsorbent to settle.

6. Do not let the column dry out; allow a small volume of eluent to remain above the column pack.
7. Use a disposable syringe or pipet to add additional solvent.

Adding the Sample

To the top of the column, add all of chromatography sample 4, gently tapping to settle the dry powder.

Chromatography of a Mixture of Ferrocene and Acetylferrocene

Eluting the Column

Two fractions will be collected. The first fraction will be eluted using hexanes. The second fraction will eluted with a 50:50 mix of hexanes and diethyl ether.

❖ To make this column chromatography a 'flash' technique

1. Use a pipet to add eluting solvent to the fill the remaining available column space.
2. Use a pipet bulb to 'push' the solvent through the column.

   Allowing only for gravity to percolate solvent through the column is very slow. The flash technique speeds up the process.
3. Create a light seal when squeezing, and break the seal before releasing pressure on the bulb.

   Be careful not to aspirate the solvent and column pack to into the pipet bulb.

   Even while pushing the solvent, always maintain the solvent level just above the column pack. Add additional solvent as needed.
4. Collect solvent in a small waste beaker as it passes through the column until the yellow band approaches the top of the cotton plug.

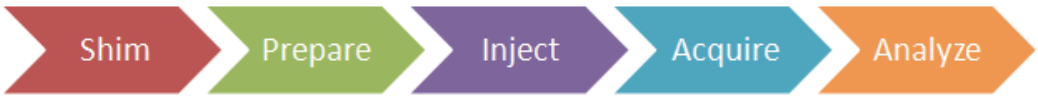
   Ferrocene (fraction 1) is eluted first with hexanes and is visible as a yellow band.
5. Collect fraction 1 in the tared, labeled vial.
6. Cap and set the vial aside.
7. Replace the waste beaker under the column.
8. Switch to the next eluting solvent.

   Acetylferrocene is eluted next (fraction 2) with a 50:50 mix of hexanes and diethyl ether, and is visible as an orange band.
9. Maintain a solvent level just above the column pack.

10. Continue collecting solvent in the waste beaker until the orange band approaches the top of the cotton plug; swap the beaker and collect this fraction in the tared vial labeled fraction 2.
11. Cap and set the vial aside.
12. Evaporate the solvents from the two vials (or flasks) and determine the weights of the crude residues.
13. **Optional**: Recrystallize fractions from a minimum quantity of hot hexanes.
14. **Optional**: Isolate and dry the crystals. Determine their weights and melting points.
15. Calculate the percent recovery of the crude and recrystallized products.

Instrumental procedure

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



Shim

Ensure the NMR spectrometer is shimmed and ready to accept samples.

Pre-sample preparation

1. Displace the shim fluid from the picoSpin capillary cartridge with air.
2. Flush the cartridge with 0.1 mL of chloroform, and then displace the solvent with an air push.

   A small signal in your sample spectrum may appear at 7.24 ppm due to residual $CHCl_3$, it can be used to shift reference the spectrum.
3. Set up the onePulse script according to parameters listed in the Pulse Script table.

Injection

1. Using a 1 mL disposable polypropylene syringe fitted with a 1½ inch long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of sample.
2. Inject about half the sample.

   Ensure all air bubbles have been displaced from the cartridge by examining the drain tube.
3. Cap both the inlet and outlet ports with PEEK plugs.

Acquire

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

Pulse Script: onePulse

Parameter	Value
tx frequency (**tx**)	proton Larmor frequency (MHz)
scans (ns)	16
pulse length (**pl**)	Instrument specific 90° pulse length
acquisition time (**aq**)	750 ms
rx recovery delay (r1)	500 µs
T1 recycle delay (**d1**)	8 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	-200 Hz
max freq. to plot	+1000 Hz
zero filling (**zf**)	8192
align-avg. data	✓
live plot	✓
JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default **pfa** values

Processing

Download the experimental JCAMP spectra files and open them by importing 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)	-

1. Import each data file into the same workspace in Mnova. Manually apply Ph0 phase correction to each spectrum.
2. Manually shift reference each spectrum using Mnova's TMS tool.
3. Assign the TMS signal (0 ppm) or $CHCl_3$ signal (7.24 ppm), whichever is present.
4. Identify and assign each signal in the spectra.
5. Save the Mnova document, print each spectrum and paste into your lab notebook.

Results

The ^1H NMR spectrum of a 0.5 M solution of ferrocene in $CDCl_3$ is presented in Figure 2a and Figure 2b. The spectrum dominated one signal centered at 4.14 ppm, due to the target sample. Due high symmetry of this metallocene, the singlet arises from excitation of 10 chemically and magnetically equivalent aromatic bis(cyclopentadienyl) protons.

Figure 2a

Full ^{1}H NMR (45 MHz) spectrum of a 0.5 M solution of ferrocene in $CDCl_3$

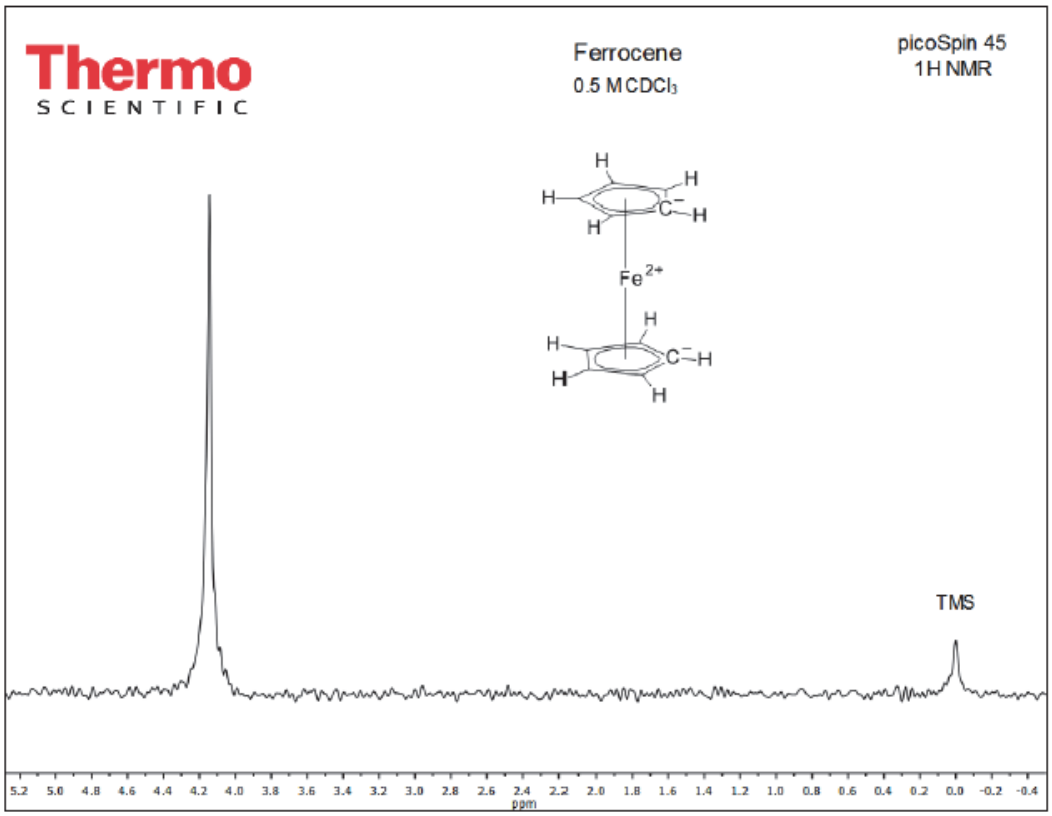
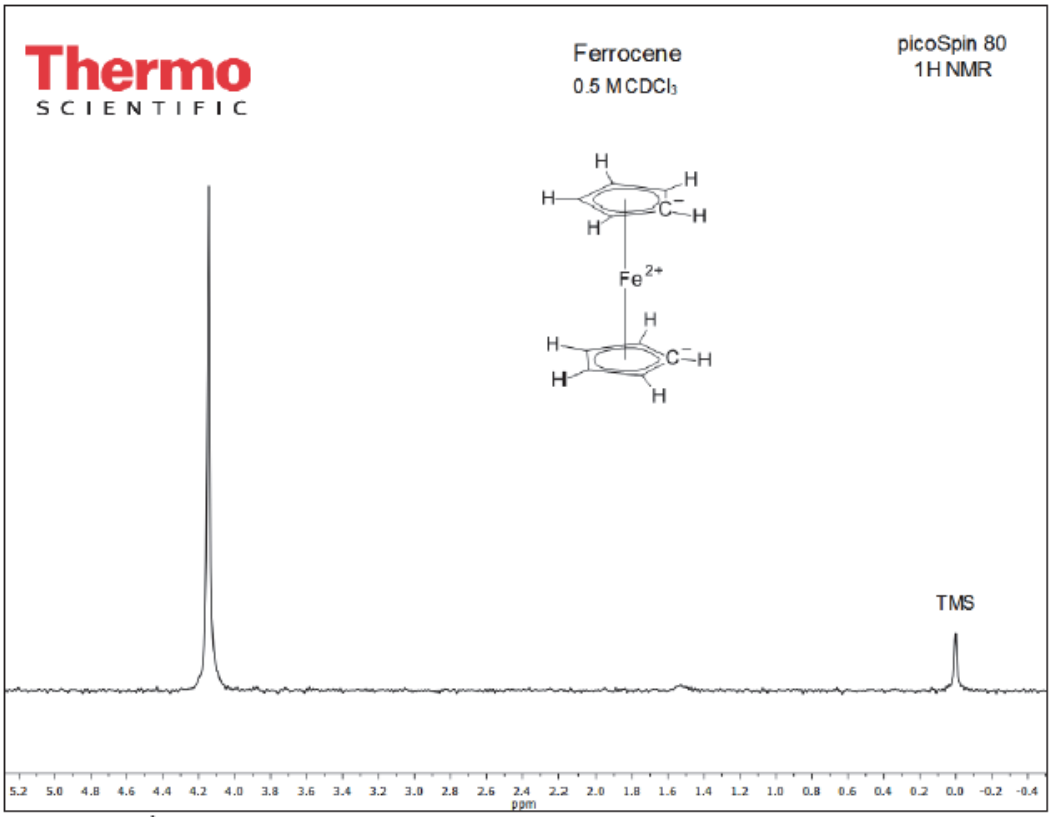


Figure 2b

Full ^{1}H NMR (82 MHz) spectrum of a 0.5 M solution of ferrocene in $CDCl_3$



The ^{1}H NMR spectrum of a 0.5 M solution of acetylferrocene in $CDCl_3$ is presented in Figure 3a and Figure 3b. Acylation of one of the aromatic cyclopentadienyl rings disrupts the previous symmetry of ferrocene, resulting in three cyclopentadienyl proton signals and one acetyl proton signal. The singlet appearing at 2.38 ppm, assigned as 'c', arises from excitation of 3 acetyl ($-CH_3$) protons. The singlet appearing at 4.19 ppm, assigned as 'b', arises from excitation of 5 protons on the unsubstituted cyclopentadienyl ring. The two multiplet signal groups appearing at 4.48 and 4.76 ppm, assigned as 'a', arise from excitation of 4 protons on the substituted cyclopentadienyl ring. The 2 ring protons closest to the acetyl group are more strongly deshielded and shift further downfield at 4.76 pm, while the other two ring protons, being more shielded, appear at 4.46 ppm. The multiple structures derive from non-first order coupling of adjacent protons on the substituted ring.

Figure 3a

Full ^{1}H NMR (45 MHz) spectrum of a 0.5 M solution of acetylferrocene in $CDCl_3$.

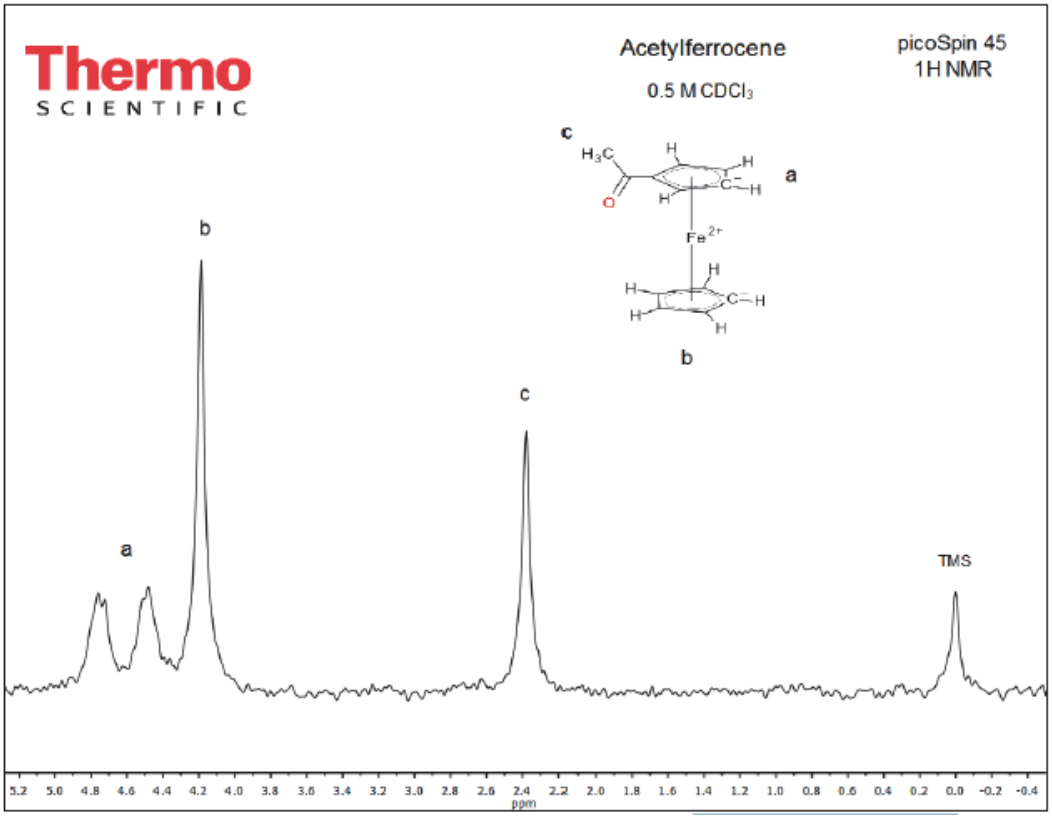


Figure 3b

Full ^{1}H NMR (82 MHz) spectrum of a 0.5 M solution of acetylferrocene in $CDCl_3$

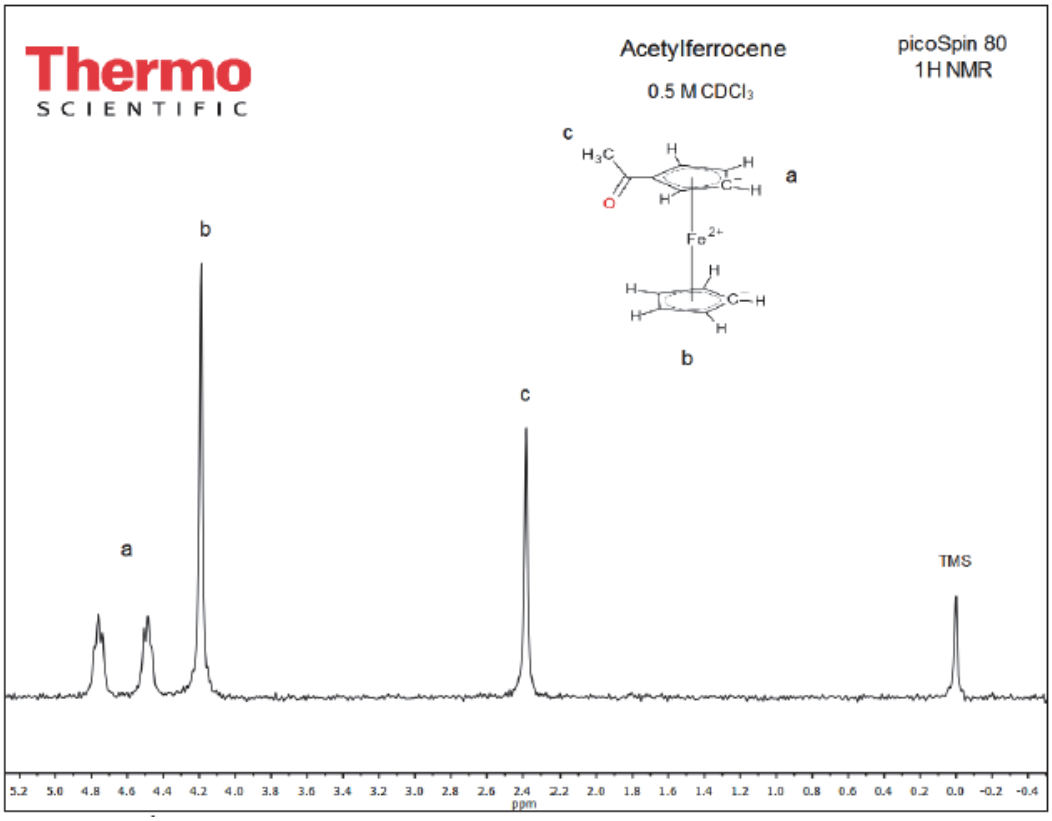


Figure 4a and Figure 4b shows the ^{1}H NMR spectrum of crude isolated reaction product, acetylferrocene, in $CDCl_3$. The characteristic acetyl singlet and double multiplet structures of the substituted ring of acetylferrocene is evident in this solution spectrum. The unsubstituted ring proton signal appears 4.19 ppm. Unreacted ferrocene appears as a shoulder peak on the upfield side (4.14 ppm) of the acetylferrocene signal. These signal groups, the double multiplet structures or acetyl group protons, aid in distinguishing acetylferrocene from ferrocene in the NMR spectrum of the mixture. Diacetylferrocene, where acetyl substitution occurs on each cyclopentadienyl ring would contain only three signals, the doublet of multiplets and 4.48 and 4.76 ppm, respectively, and an acetyl signal at 2.38 ppm. It is distinguishable from acetylferrocene by the absence of the unsubstituted cyclopentadienyl signal. Integrating the spectrum can reveal the presence diacetylferrocene. The expected signal ratio for acetylferrocene is 2:2:5:3, deviation from this ratio suggests the presence of diacetylferrocene.

Figure 4a

Full ^{1}H NMR (45 MHz) spectrum of a 0.5 M solution of crude reaction product (acetylferrocene) in $CDCl_3$ prior to column purification

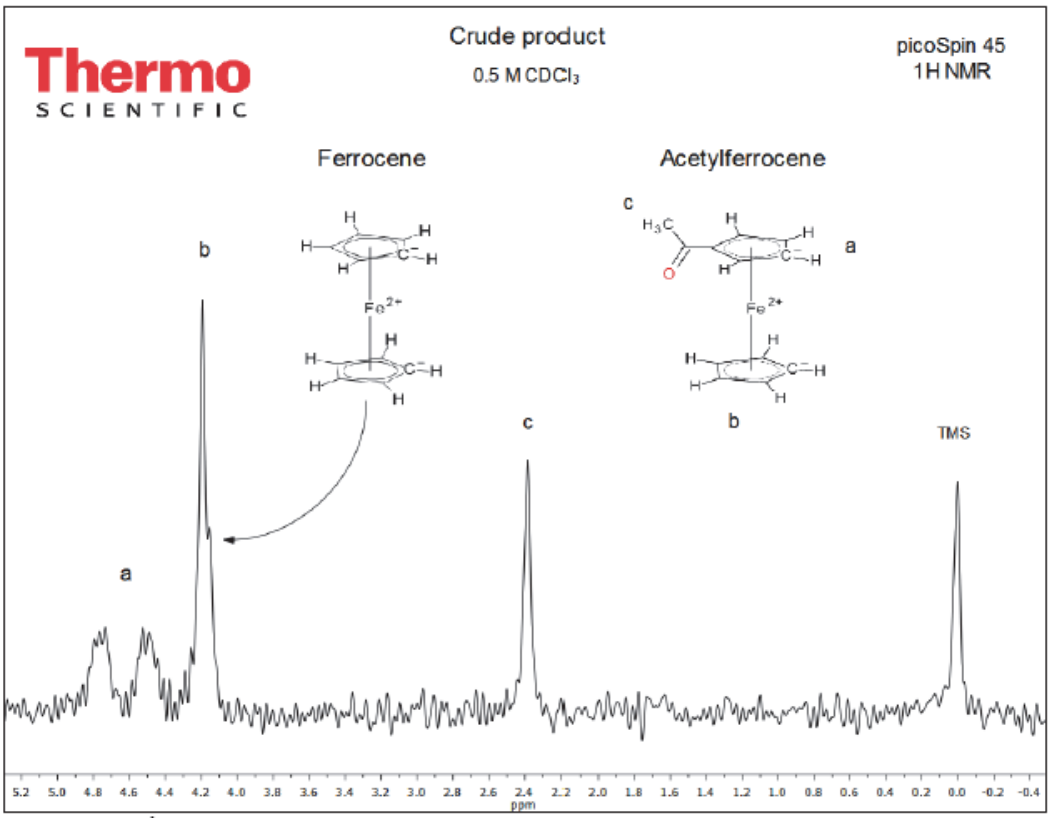
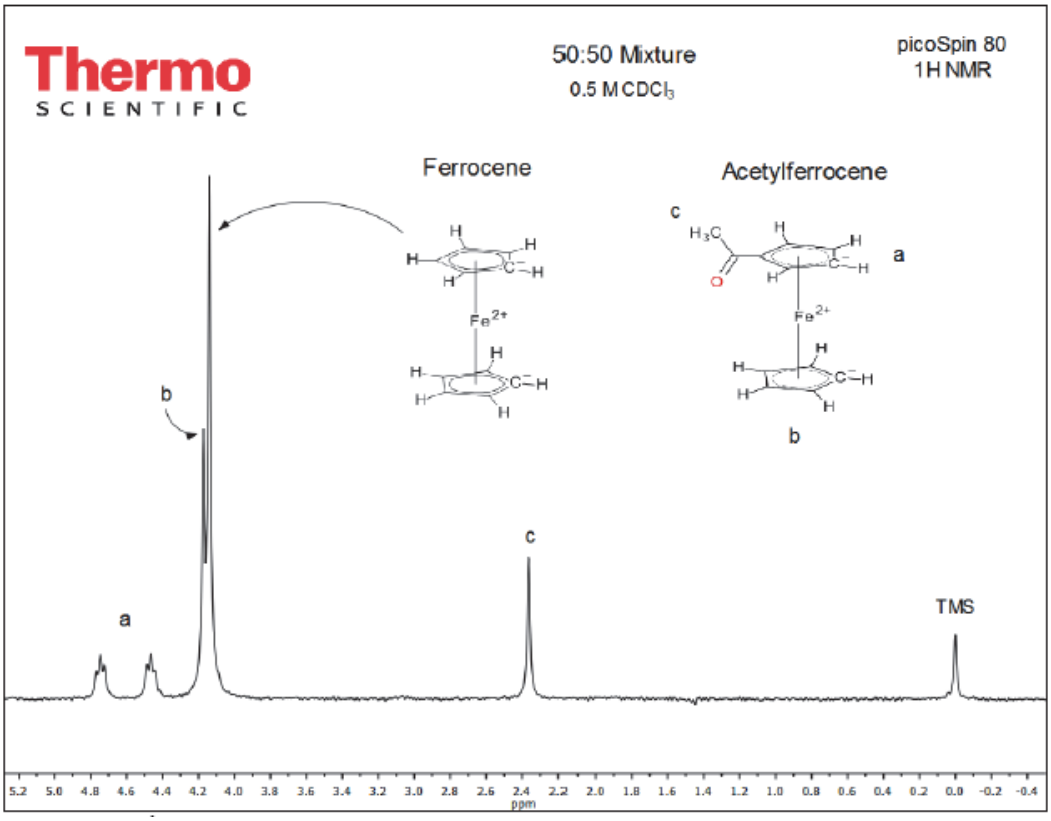


Figure 4b

Full ^{1}H NMR (82 MHz) spectrum of a 0.5 M solution of a 50:50 mixture of ferrocene and acetylferrocene in $CDCl_3$



The NMR spectrum isolated and purified product eluted from the chromatographic column, fraction 2, and recrystallized is shown in Figure 5. Similar to the crude product spectrum (Figure 4a and Figure 4b), this spectrum displays the characteristic double multiplet structures of acetylferrocene and the acetyl singlet at 2.38 ppm. The shoulder peak due to ferrocene on the upfield side of the unsubstituted cyclopentadienyl ring of acetylferrocene signal is visibly absent as expected after the product is chromatographed on the silica gel column.

Table 1 ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
2-5	TMS	$Si(CH_3)_4$	0	12 H	Singlet
2, 4, 5	Ferrocene	$Fe[C_5H_5]_2$	4.14	10 H	Singlet
3-5	Acetylferrocene	$Fe[C_5H_5]C_5H_4C(O)CH_3$	2.38	3 H	Singlet
		$Fe[C_5H_5]C_5H_4C(O)CH_3$	4.19	5 H	Singlet
		$Fe[C_5H_5]C_5H_4C(O)CH_3$	4.48	2 H	Triplet
		$Fe[C_5H_5]C_5H_4C(O)CH_3$	4.76	2 H	Triplet
	Water	HOD	4.65	1 H	Singlet
	Chloroform	$CHCl_3$	7.24	1 H	Singlet
	Acetone	$O=C(CH_3)_2$	2.05	6 H	Singlet

Comments

The challenges of this lab experiment are manifold and largely related to preparing and using a microscale column.

- Preparation of a microscale column in a Pasteur pipet – dry packing is preferred.
- Maintaining adequate solvent volume above the column pack, while applying pressure with a pipet bulb.
- Small headspace above the column pack after adding the pre-adsorbed mixture of alumina and then solvent.
- Evaporating off relatively large quantities of eluting solvent.

The volume of ice water (1 mL) and sodium hydroxide (~3 mL) added to the reaction tube takes the total liquid volume close to the top of the tube, making it difficult to mix solution. A glass stir rod is helpful to mix the solution.

Own Observations

Ordering Information

To reorder this lesson plan from the *picoSpin NMR Spectroscopy: Example Lesson Plans* set, please refer to document order number: "**LP52592_E 05/14M** picoSpin Lesson Plan #4-Friedel-Crafts Acylation of Ferrocene"

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Note Please have the instrument serial number available when you contact us.

Simple Distillation of a Toluene-Cyclohexane Mixture: picoSpin 45 or picoSpin 80

Introduction

There are four basic distillation techniques for separating and purify the components of a liquid mixture: simple distillation, fractional distillation, vacuum distillation and steam distillation. The chosen distillation method and extent of purification will depend on the nature of the mixture, specifically the difference of the boiling points of miscible liquids. In distillation, the mixture is heated, vaporizing a substance. Under boiling reflux, the vapor phase becomes richer in the lower boiling component as vapors continue to condense and move up the distillation head, purifying the mixture.

Simple distillation is most effective when applied to mixtures where the liquid components differ in their boiling points by at least 50 °C. As the first component distills, the temperature is measured from vapor it condensing on the bulb of a thermometer positioned just below the sidearm of the distilling head. With simple distillation, the rate of change of temperature is a slow as the composition of the boiling liquid changes as distillation progress. Thus, the range over which liquid is purified is not sharp. The temperature of the distilling liquid is observed to plateau and then drop before rising again, as the process of distilling the second component begins. Here, the temperature will plateau near the boiling point of the second lowest boiling liquid in the mixture, thus distilling the second fraction. The process continues for each subsequent component, leaving the highest boiling liquid in the distilling flask. By carefully controlling the rate of distillation, it is possible to affect reasonably good separation. If distillation is rapid, then separation of the components of mixture is poorer than if the mixture is distilled slowly.

Purpose

The purpose of this experiment is to separate components of a mixture using traditional simple distillation. A miscible liquid mixture is heated in a round bottom flask fitted with a distilling head, thermometer and condenser. The large surface area of the heating flask allows for transfer of sufficient thermal energy to distill components of a mixture. Under typical boiling conditions, as the solution is heated, equilibrium develops between the vapor and liquid phase, separating out in the vapor phase the lower boiling component. By distilling too rapidly, added heat and excess vapors disrupts the equilibrium, causing higher-boiling

components to distill in early fractions. As the distillation proceeds, the condensation line moves up the cold surface of the flask, heating it and distilling the first component. Reaching the thermometer bulb the vapor-phase temperature is measured just before it condenses and liquefies in an air or water-cooled condenser tube. Condensed, purified liquid then flows to a collection flask.

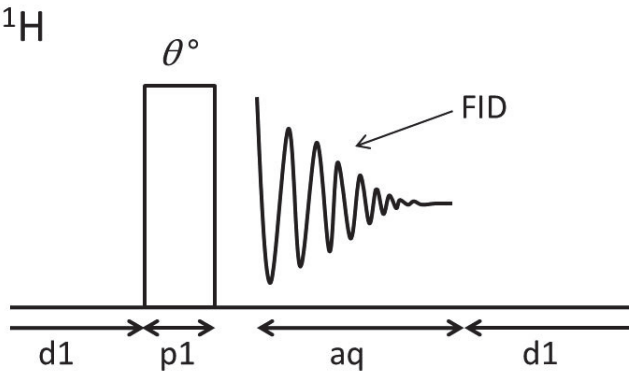
In the experiment, a 50:50 mixture of cyclohexane and toluene will be distilled, separating the lower boiling component for the mixture. The initial mixture, the distillate and the pot reside will be analyzed using the Thermo Scientific™ picoSpin™ 45 or picoSpin™ 80 NMR spectrometer. Samples will be quantified but integrating resonance signals in the spectra to determine the molar ratio of the initial mixture, distillate and pot residue, and to evaluate the efficiency of simple distillation of our choice of liquid samples.

Literature

Adapted from Williamson, K. L.; Minard, R.; Masters, K. M. *Macroscale and Microscale Organic Experiments*, 5th ed., Houghton Mifflin Co., **2007**.

Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



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 tim (ms)
ns: # of scans (individual FIDs)

Procedure and Analysis

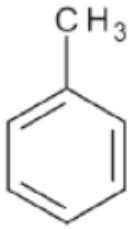
Time requirements: 2 hours
Difficulty: Easy
Sample: Cyclohexane, toluene

Equipment/materials:

- picoSpin 45 or picoSpin 80
- Thermometer

- Cyclohexane (C6H12)
- Toluene (C5H5CH3)
- Tetramethylsilane (TMS; (CH3)4Si)
- Simple distillation apparatus
  - 100 mL round bottom flask
  - 25 mL Erlenmeyer flask
  - Condenser
  - Three-way adapter
  - Vacuum adapter
  - Clamps (flask or Keck)
  - Ring stand, ring clamp, iron ring
- Thermometer adapter
- Boiling chips
- Mnova NMR Processing Suite
- picoSpin accessory kit:
  - Port plugs
  - Syringe port adapter
  - Drain tube assembly
- 25 mL beaker
- 1 mL polypropylene syringes
- 22 gauge blunt-tip dispensing needles
- 2 and 7 mL vials

Molecules



Toluene


Cyclohexane

Physical data

Substance	FW (g/mol)	Quantity	MP (°C)	BP	Density (g/mL)
Toluene	92.14	10 mL	-95	111	0.8669
Cyclohexane	84.16	10 mL	6.47	80.74	0.779
Tetramethylsilane (TMS)	88.22	3 drops	-99	26-28	0.648
Chloroform-d ($CDCl_3$) w/1%TMS	120.384	1 mL	-64	61	1.50
Acetone-d_6 (Ac-d_6) w/1%TMS[a]	64.12	1 mL	-94	56	0.872

[a] Optional NMR solvents

Safety Precautions

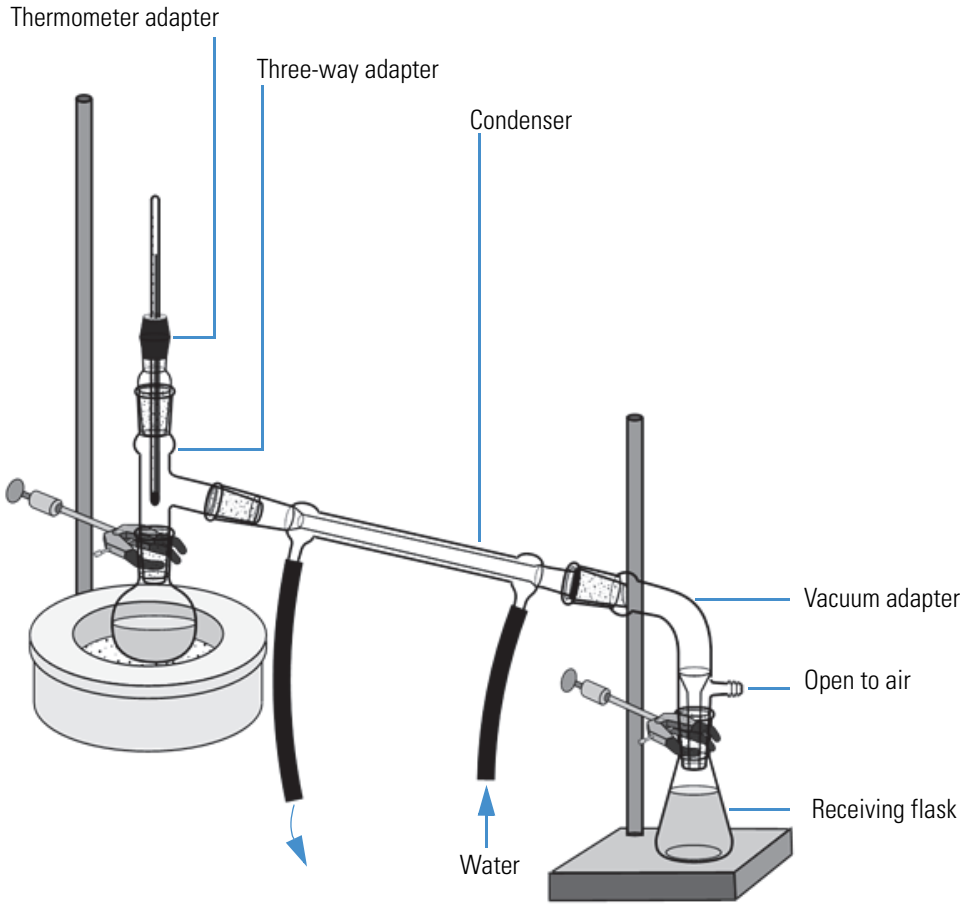
NOTICE Be sure that all persons operating this system read the site and safety manual first.

Experimental Procedure

Reaction procedure

1. Set up a simple distillation apparatus.

Simple distillation apparatus



   Use a sand bath as a heat source.

2. To a 50 mL round bottom flask, add approximately 10 mL of toluene, 10 mL of cyclohexane, and a boiling chip.
3. Swirl the mixture then take a 0.25 mL aliquot for Sample 3 and transfer it to a 2 mL vial.
4. Place the thermometer bulb so it reaches below the sidearm of the three-way adapter.
5. Water-cool the condenser.
6. Place a receiving vial at the outlet of the vacuum adapter.
7. Place the vial in a 25 mL beaker filled with ice.
8. Control heating of the round bottom flask by piling up hot sand.

As distillation begins, vapors will rise and condense on the cold glass.

9. Control the boiling rate by removing some sand so that only about 2 drops per minute is collected in the receiving flask.
10. Record the temperature as the first drops of liquid are collected.

    This temperature reflects the boiling point of the cyclohexane distillate.
11. Collect distillate until distillation of cyclohexane stops and the observed temperature drops (approximately 7 mL).

    Do not distill to dryness.
12. Turn off the sand bath.
13. Prepare samples for NMR analysis

Preparing Samples

Several samples will be prepared for analysis. The samples can be analyzed and neat samples. Since the ^{1}H NMR chemical shifts of toluene and cyclohexane are well known, we can use their signals as an internal chemical shift reference. Alternatively, a few microdrops of TMS (0 ppm) can be added to the test samples. The sample preparation guide and spectra presented are for neat samples with added TMS.

Sample 1: To a labeled vial measure about 0.20 mL of toluene, add a couple microdrops of TMS. Cap and save for NMR analysis.

Sample 2: To a labeled vial measure about 0.20 mL of cyclohexane, add a couple microdrops of TMS. Cap and save for NMR analysis.

Sample 3: To a labeled vial measure about 0.20 mL of the initial mixture of toluene and cyclohexane from the round bottom flask, add a couple microdrops of TMS. Cap and save for NMR analysis.

Sample 4: To a labeled vial measure about 0.20 mL of distillate from the receiving flask, add a couple microdrops of TMS. Cap and save for NMR analysis.

Sample 5: To a labeled vial measure about 0.20 mL of distillate from the round bottom flask, add a couple microdrops of TMS. Cap and save for NMR analysis.

Instrumental Procedure

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

Shim

Ensure the NMR spectrometer is shimmed and ready to accept samples.

Presample preparation

1. Displace the shim fluid from the picoSpin capillary cartridge with air.
2. Flush the cartridge with 0.1 mL of chloroform, and then displace the solvent with an air push.

   A small signal in your sample spectrum may appear at 7.24 ppm due to residual chloroform, it can be used to shift reference the spectrum.
3. Set up the onePulse script according to parameters listed in the Pulse Script table.

Injection

1. Using a 1 mL disposable polypropylene syringe fitted with a 1½ inch long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of sample.
2. Inject about half the sample.

   Ensure all air bubbles have been displaced from the cartridge by examining the drain tube.
3. Cap both the inlet and outlet ports with PEEK plugs.

Acquire

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

Pulse Script: onePulse

Acquisition parameters apply to both the picoSpin 45 and picoSpin 80 spectrometers. Use the Tx frequency (tx) and pulse length (p1) appropriate for each system.

Parameter	Value
tx frequency (**tx**)	proton Larmor frequency (MHz)
scans (**ns**)	4
pulse length (**pl**)	Instrument specific 90° pulse length
acquisition time (**aq**)	750 ms
rx recovery delay (**r1**)	500 µs
T1 recycle delay (**d1**)	6 s
bandwidth (**bw**)	4 kHz

Parameter	Value
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	-200 Hz
max freq. to plot	+1000 Hz
zero filling (**zf**)	8192
align-avg. data	✓
live plot	✓
JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default **pfa** values

Processing

Download the experimental JCAMP spectra files and open them by importing 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 Hz
First Point	0.5
Shift reference (CS)	Manually reference
Peak Picking (pp)	Manually select peaks

Function	Value
Integration (I)	Automatic Selection
Multiplet Analysis (J)	-

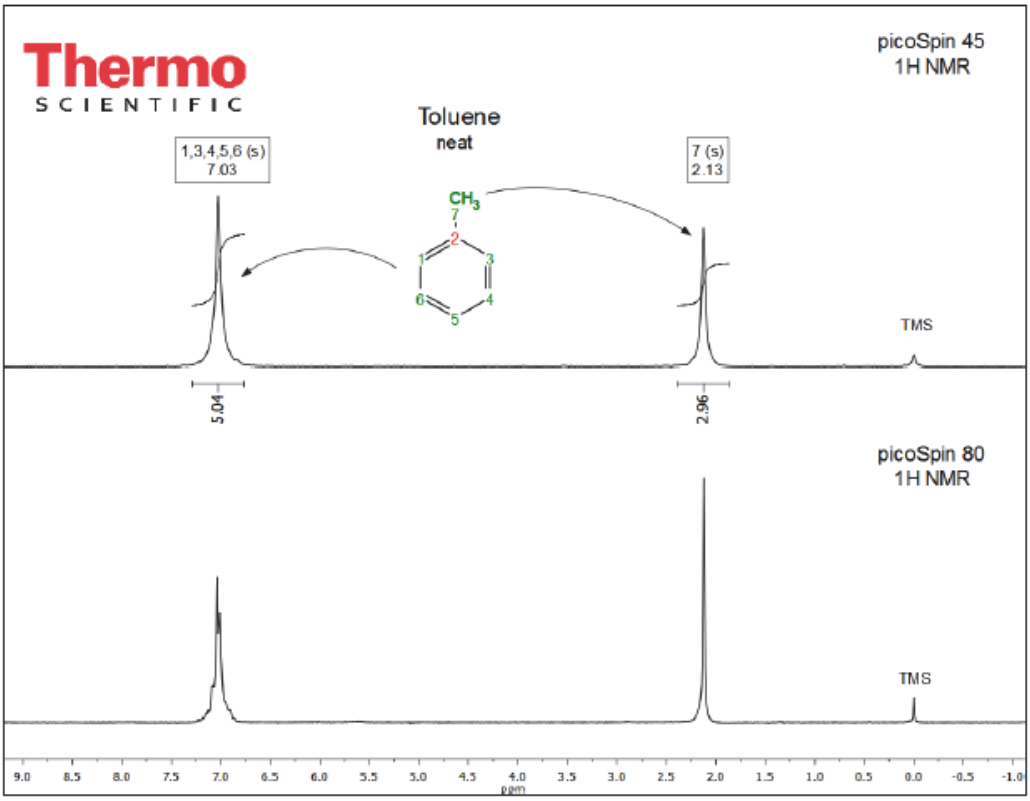
1. Import each data file into the same workspace in Mnova.
2. Manually apply Ph0 phase correction to each spectrum.
3. Manually shift reference each spectrum using Mnova's TMS tool.
4. Assign the TMS signal (0 ppm), residual chloroform signal (7.24 ppm), cyclohexane signal (1.38 ppm), or toluene signals (2.09 or 6.98 ppm).
5. Identify and assign each signal in the spectra.
6. Integrate each signal group associated with toluene and cyclohexane, and determine the relative molar concentrations of each component of the initial mixture, distillate and pot reside.
7. Save the Mnova document, print each spectrum and paste into your lab notebook.

Results

The 45 MHz and 82 MHz ^{1}H NMR spectrum of toluene (neat) is presented in Figure 2. The spectrum two signals, one downfield signal centered at 7.00 ppm due to five aromatic protons (C_6H_5), and a second upfield at 2.09 ppm arising from excitation of the methyl group ($-CH_3$) attached to the phenyl ring. The methyl protons do not coupling owing to the lack of neighboring protons. Spin-spin coupling isn't observed in the aromatic ring protons of toluene in the 45 MHz spectrum, but at 82 MHz the slight differences in the chemicals shift position of ortho (7.04 ppm), meta (7.08 ppm) and para (7.01 ppm) protons are observable (Figure 3). Integration of the signals reveals an expected 5:3 proton ratio.

Figure 2

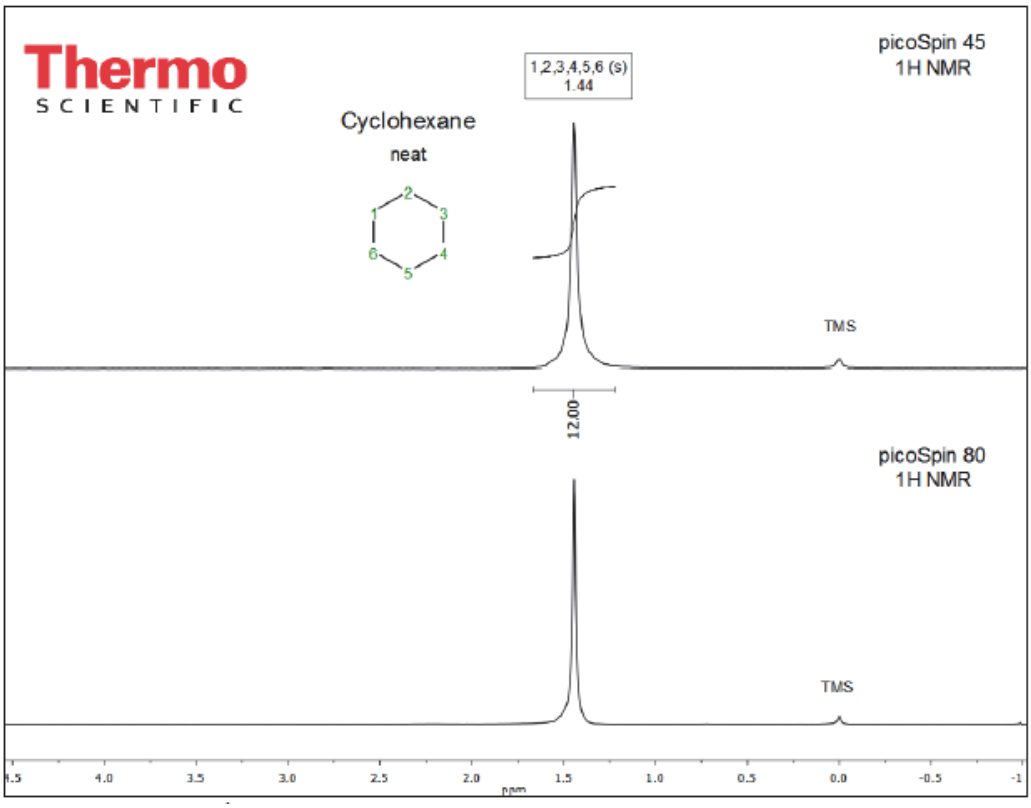
Stacked, full ^{1}H NMR 45 MHz (top) and 82 MHz (bottom) spectrum of toluene (neat) with TMS



Shown in Figure 3 are the 45 MHz (top) and 82 MHz (bottom) spectra of neat cyclohexane. The spectrum is dominated by single resonance due to 12 equivalent protons in for the cyclohexane appearing at 1.44 ppm. Integrating the peak yields an expected integral value of 12.

Figure 3

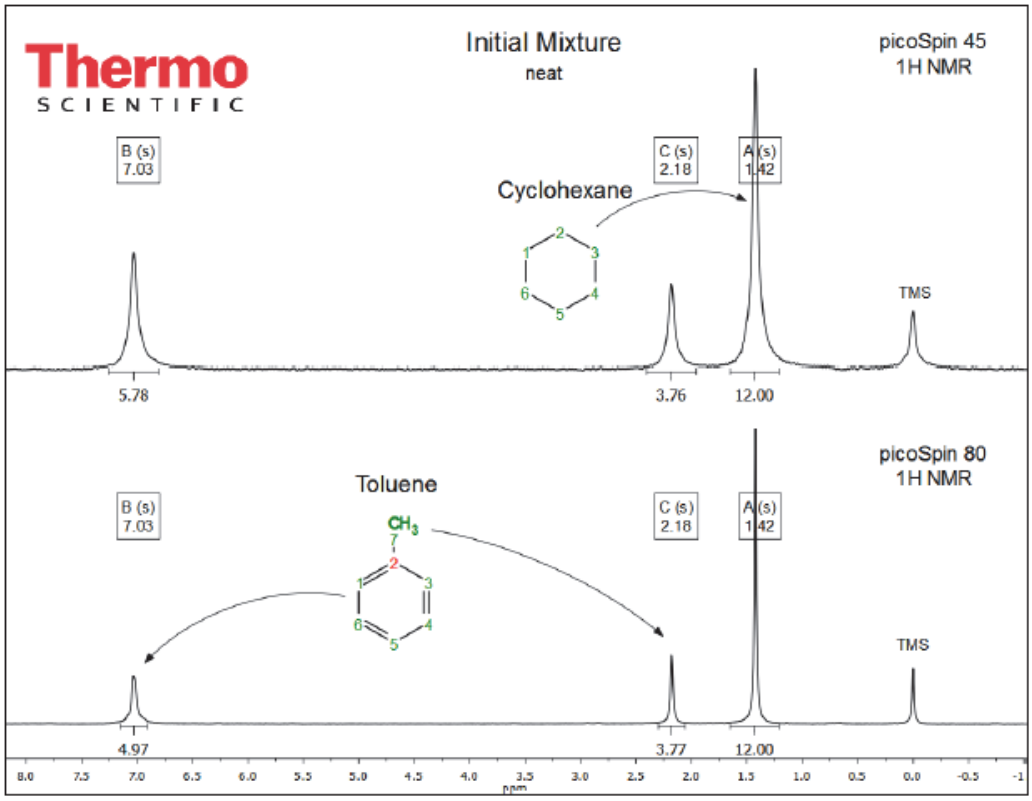
Stacked, full ^{1}H NMR 45 MHz (top) and 82 MHz (bottom) spectrum of a cyclohexane (neat) with TMS



In Figure 4, we see the first spectrum of our mixture; it shows both the 45 MHz (top) and 82 MHz (bottom) ^{1}H NMR spectrum of the initial mixture of toluene and cyclohexane. The signals attributable to each species are identified in the spectra. Initially, we measured roughly equal volumes of the two compounds. Looking at the 45 MHz spectrum, if we integrate individual signals and normalize the cyclohexane signal to 12, due to its 12 chemically equivalent protons, we obtain an integral ratio of 5.78:3.76:12.00. Were the sample exactly a 50:50 mixture of the two components we would expect a ratio of 5:3:12. To estimate the molar ratio between the two compounds, compare the integration of the methyl group in toluene (3.76 at 2.24 ppm) to the integration of the cyclohexane signal (12 at 1.44 ppm); the ratio is 3.76:12. Normalizing this ratio to account for 3 protons in the methyl group of toluene and 12 protons in cyclohexane, we get a ratio of 1.25:1, which gives us a molar ratio of 55% toluene to 45% cyclohexane, very close to the intended 50:50 ratio. We can apply the same approach using the aromatic signal but then its relative integral value would need to be normalized for the 5 protons its signal contains. We can also evaluate the 82 MHz spectrum in the same way.

Figure 4

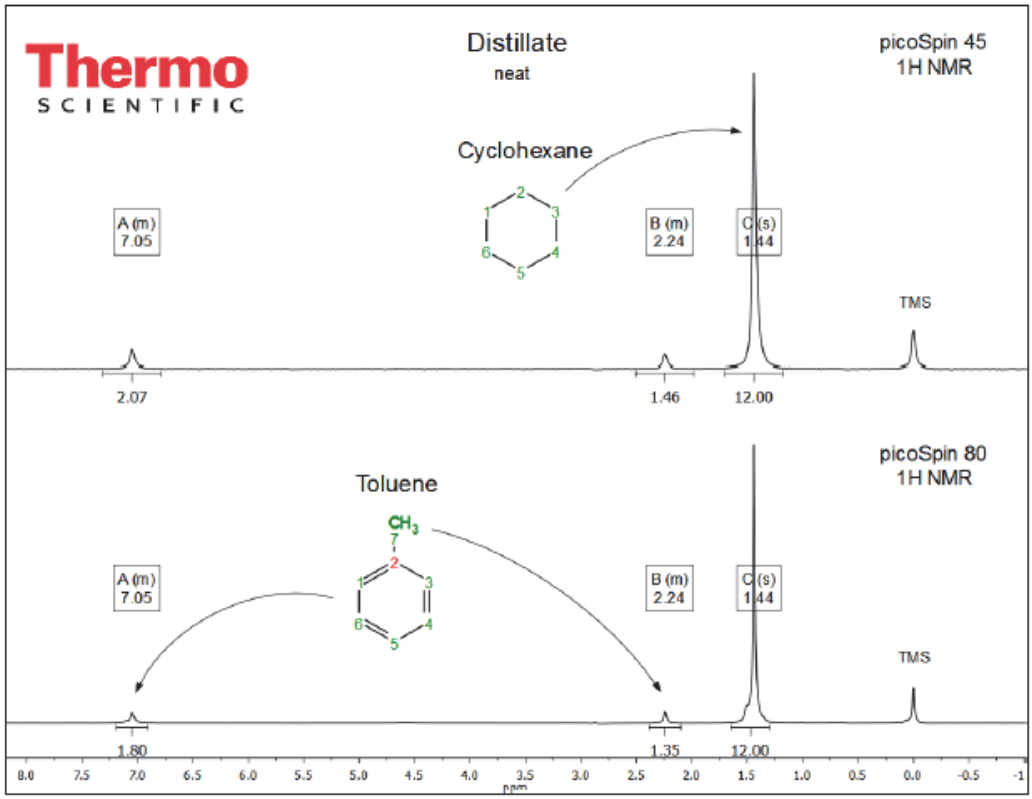
Stacked, full ^{1}H NMR 45 MHz (top) and 82 MHz (bottom) spectra of the initial distillation mixture with TMS



The NMR spectrum of the distillate is shown in Figure 5. The cyclohexane signal is easily identifiable as the largest signal at 1.44 ppm. Qualitatively, the toluene signals appear considerably smaller when compared to their size in the initial mixture spectrum. However, a quantitative measure will give a better estimate of molar ratio of components in the distillate. Applying the same approach, we compare the relative ratio of the methyl proton signal from toluene to that of cyclohexane and we obtain 1.46:12. Normalizing for the number of protons comprising each signal we get a normalized ratio of 0.49:1 from the 45 MHz spectrum. This yields an estimated molar ratio of 33% toluene to 67% cyclohexane. Visually the minor species looks considerably smaller than the 33% just calculated. However, there are 12 protons in the cyclohexane signals whereas there are only 3 in the methyl group signal, and it is difficult to visually normalize these differences.

Figure 5

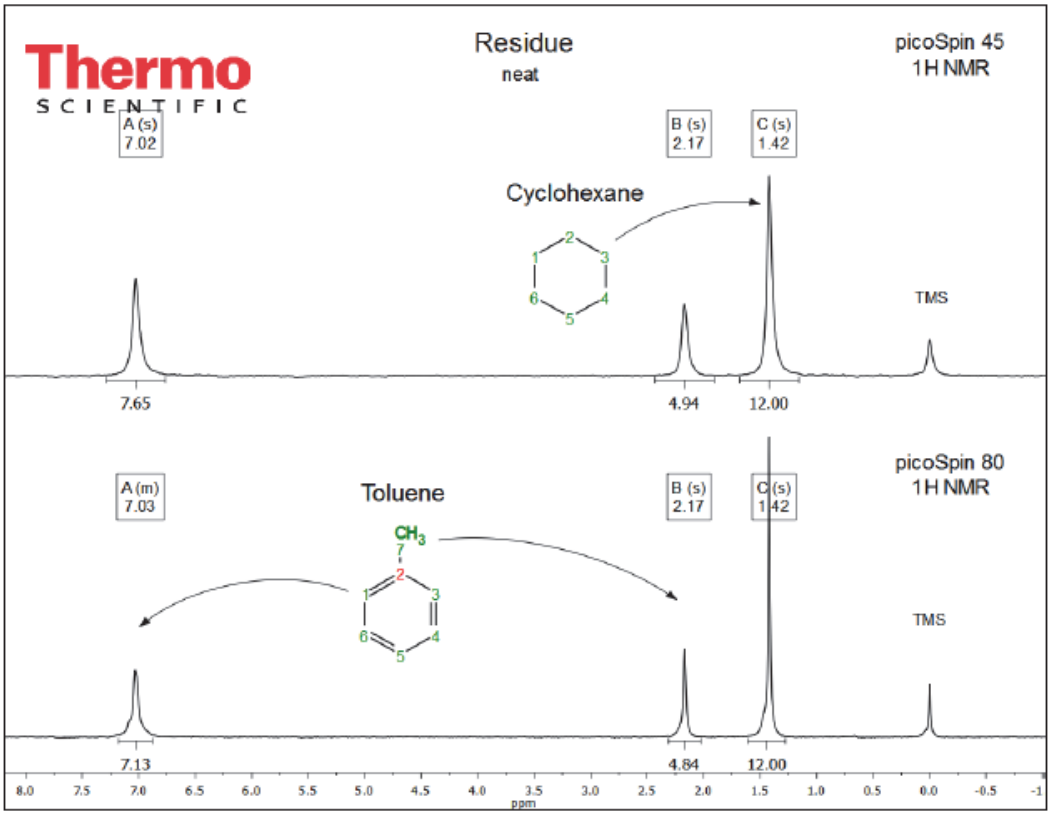
Stacked, full ^{1}H NMR 45 MHz (top) and 82 MHz (bottom) spectra of distillate with TMS



Finally, we evaluate the pot residue; its spectrum is presented in Figure 6. Here we see a spectrum very similar to those in Figures 4 and 5; signals attributed to toluene (7.00 ppm and 2.38 ppm) and cyclohexane (1.44 ppm) are easily identifiable.

Figure 6

Stacked, full ^{1}H NMR 45 MHz (top) and 82 MHz (bottom) spectra of distillate with TMS



Conclusions

Simple distillation is effective for mixtures where the components have a boiling point difference of about 75 °C; the boiling points of our sample are 88 °C and 111 °C for cyclohexane and toluene, respectively. Owing to the closely spaced boiling points, simple distillation is less effective at separating the two components and we expect to see a higher concentration of toluene in the vapor phase condensing along with cyclohexane. Quantitative analysis of the 45 MHz spectrum of the residue yields a normalized molar ratio of 1.65:1, or 62% toluene to 38% cyclohexane.

Comments

This lab offers no specific challenges.

Table 1 ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
2-6	TMS	$Si(CH_3)_4$	0	12 H	Singlet
2, 4-6	Toluene	$C_6H_5CH_3$	2.24	3 H	Singlet
		$C_6H_5CH_3$	7.08(*m*), 7.04(*o*), 7.01(*p*)	5 H	Singlet
3-6	Cyclohexane	C_6H_{12}	1.44	3 H	Singlet
	Chloroform	$CHCl_3$	7.24	1 H	Singlet
	Acetone	$O=C(CH_3)_2$	2.05	6 H	Singlet

Own Observations

Ordering Information

To reorder this lesson plan from the *picoSpin NMR Spectroscopy: Example Lesson Plans* set, please refer to document order number: "**LP52593_E 05/14M** picoSpin Lesson Plan #5-Simple Distillation of a Toluene-Cyclohexane Mixture"

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