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picoSpin 45 Proton Nuclear Magnetic Resonance Transesterification Reaction Monitoring

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Introduction

Recently developed compact Nuclear Magnetic Resonance (NMR) spectrometers open new possibilities in Process Analytical Technology (PAT) and quality assurance/quality control applications. NMR is a non-destructive analytical technique providing structural and quantitative chemical information, typically under static sample conditions. However, NMR analysis can also be applied to monitoring dynamic changes during the course of a chemical reaction.

Compact instrumentation like the Thermo Scientific™ picoSpin™ 45 proton (^{1}H NMR) spectrometer introduces a capillary cartridge probe design, providing chemists a flow path from reaction vessel to the spectrometer. Incorporating a radio frequency (RF) micro-coil technology in the probes design also reduces the sample volume needed for analysis. As a PAT tool, this design feature is particularly attractive for batch reaction monitoring and online process stream applications because the technology offers low cost and compact instrumentation which is adaptable to current workflows.

In this article, we illustrate how a reaction can be monitored by 45 megahertz (MHz) ^{1}H NMR by examining acid catalyzed transesterification reaction of Ethyl Acetate (EtOAc) to Methyl Acetate (MeOAc).

Methods

The experiments were conducted using the picoSpin 45 NMR compact spectrometer.[1] The spectrometer is a 45 MHz pulsed, Fourier transform ^{1}H NMR permanent magnet instrument. The spectrometer's capillary cartridge is fitted with micro-fluidic inlet and outlet connectors on the front panel. The fluid path between them is a capillary with an internal diameter of about 0.3 mm and a total volume of about 20 microliters (µL). Liquid samples are introduced into the spectrometer's RF coil either by manual or automated injection. In this experiment, manual sample injection was performed.

A solution of Methanol (MeOH) and EtOAc (6:1 mol/mol) was prepared in a 10 mL high-density polyethylene (HDPE) bottle by mixing 5 mL of dry MeOH (Sigma-Aldrich: 99.8%; 24.70 M) with 2 mL of EtOAc (Acros: 99.6%; 10.24 M). To start the reaction, 7 drops (0.3 mL) of concentrated sulfuric acid (H_2SO_4) (18.40 M, 0.75 M after dilution) was added to the reaction mixture. The solution was shaken briefly, transferred to a 13 mm × 100 mm test tube and placed in a hot water bath pre-heated to 56 °C. The reaction vessel was capped with a loose fitting glass stopper to minimize evaporation of reactants and products.

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A 40 µL aliquot of the reaction mixture was taken at a sampling interval of 5 minutes, from t = 0 to t = 180 minutes, and injected into the inlet port of the capillary probe. The magnet temperature was 46 °C and approximates the capillary temperature within the spectrometer's RF coil. After an elapsed time of 180 minutes, the reaction mixture was transferred to a HDPE bottle, sealed and allowed to react for an additional 40 hours, at which time a final spectrum was acquired. A spectrum of the unreacted MeOH:EtOAc solution, prior to the addition of acid, was also acquired for comparison. A 40 µL sample volume provided sufficient material to purge the capillary of the previous analyte, filling the RF coil volume new sample.

In situ reaction monitoring was performed on a single 40 µL aliquot of 1.4 mL reaction mixture of MeOH (1 mL) and EtOAc (0.4 mL) with 2 drops of concentrated H_2SO_4 added as a catalyst. The reaction temperature was held at a magnet temperature of 46 °C.

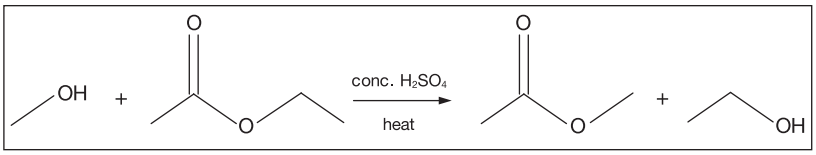

Figure 1: Acid catalyzed transesterification reaction of MeOH with EtOAc

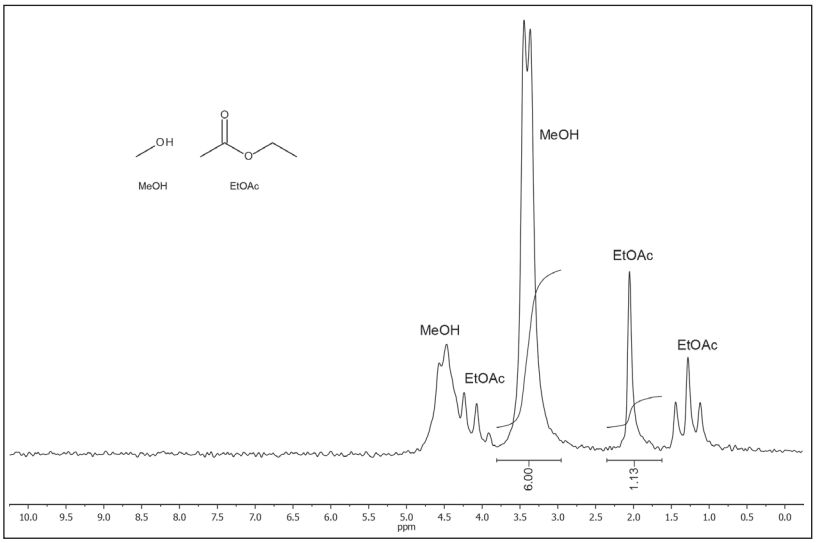

Figure 2: NMR spectrum of the initial, uncatalyzed reaction mixture; 6:1 (mol/mol), MeOH and EtOAc exhibiting hydrogen bonding effect on spin coupling in methanol

Data Processing

Each spectrum is an average of 10 or 12 scans acquired using 90° pulse, 750 ms acquisition time and 3, 5 or 10 seconds recovery delay. Averaged spectra were processed in Mnova™ NMR analysis software program, by applying automatic base-line correction, manual phase correction (PH0 and PH1), and zero filling to 64 K points; spectra were filtered using Sine Bell (26°), exponential (0.10 Hz) and Gaussian (1 Hz) apodization.[2]

Spectra were internally referenced against the methyl (-CH_3) ^{1}H signal in MeOH which was set to a chemical shift of 3.48 parts per million (ppm). Spectra were presented as unnormalized except where noted. Peak areas were obtained with Mnova's integration function for all spectra over the same spectral range for the -CH_3 signal in MeOH (3.255 to 3.619 ppm) and acetyl (α-CH_3) signal in EtOAc and MeOAc (1.927 to 2.257 ppm).

Results

The acid catalyzed transesterification reaction of EtOAc to MeOAc was monitored using the picoSpin 45 NMR spectrometer (Figure 1).

Figure 2 shows the ^{1}H spectrum of unreacted MeOH:EtOAc solution. MeOH produces two singlet peaks, i.e. -CH_3 peak at 3.48 ppm and Hydroxyl (-OH) peak at 4.70 ppm. EtOAc produces three signals as follows: triplet (1.36 ppm) arising from the terminal -CH_3 being split by two vicinal ^{1}H on the adjacent methylene (-CH_2), a singlet at 2.13 ppm from excitation of acetyl -CH_3 protons, and a quartet centered near 4.22 ppm (this signal is partially masked by -OH signal of MeOH where only three peaks of this quartet are discernible). Integration of the -CH_3 signal from MeOH and that of the acetyl methyl from EtOAc approximates the 6:1 mole ratio of the original solution.

An additional structure in both -CH_3 and -OH proton signals of MeOH is observed. The doublet signal at 3.48 ppm has J-value of 3.9 Hz and suggests that -CH_3 protons are split by the adjacent -OH proton. Ordinarily proton exchange is too rapid for spin coupling to be observed in MeOH. The observed splitting is the result of hydrogen bonding with EtOAc which slows the proton exchange rate, thus increasing the lifetime of isolated -OH protons allowing for spin-spin coupling to occur. This behavior is known to occur in MeOH solutions containing sufficient concentration of a ketone to encourage hydrogen bonding.

In Figure 3, a composite image of spectra acquired during the reaction taken at 5 minute intervals, from t = 0 to t = 180 minutes, is presented. Several regions of the composite spectrum are expanded to provide clarity and to emphasize changes occurring in acetyl, ethyl and -CH_3 ester, -CH_2 and -OH signals.

The peak centered at 2.13 ppm is attributable to a resonance of acetyl protons in both the reactant, EtOAc, and product, MeOAc. Since chemical transformation occurs on the ester side of EtOAc and the reaction does not create any new keto methyl groups, the chemical shift of these α-carbon protons is not expected to change during the course of reaction.

Initially, the triplet structure at 1.35 ppm is due to splitting of the terminal alkoxy -CH_3 group in EtOAc being split by two vicinal protons on the adjacent -CH_2 group. As the reaction progresses, the triplet signal shifts upfield to 1.29 ppm and is attributable to splitting of the -CH_3 group in ethanol (EtOH).

The signal centered on 3.76 ppm appears to be a triplet but is actually two overlapping signals: one a quartet due to splitting of -CH_2 group by three vicinal protons of the terminal -CH_3 group of the product EtOH, while the second signal comes from the alkoxy -CH_3 group of the product MeOAc. Only three of the four peaks of the EtOH methylene quartet are resolved, with the high frequency peak buried under the leading edge of the large -CH_3 signal from MeOH (3.48 ppm). The alkoxy -CH_3 proton resonance overlaps nearly perfectly with the third downfield peak of the -CH_2 quartet giving the appearance of triplet structure. There are no triplet resonances expected to appear in this region of the spectrum.

The progress of the reaction is best observed in the downfield 'walk' of the -OH signal, indicating a change in the mole fraction of MeOH and EtOH as the reaction proceeds. Initially, the reaction contains only MeOH, with MeOH:EtOH mole fraction of 6:1. As EtOH is generated, the time averaged -OH signal arising from a rapid exchange of -OH protons shifts to higher frequency as the MeOH:EtOH mole ratio changes. The -OH signal reaches its maximum downfield chemical shift at t = 180 minutes. Comparison of a spectrum taken of the reaction mixture at t = 40 hours (Figure 4) to that obtained at t = 180 minutes suggests the reaction has already finished by this time.

Close examination of the composite spectrum (Figure 3) suggests the reaction is nearly complete in the first 20–30 minutes of reaction. Figure 5 shows the first six spectra taken at time intervals of t = 0 to t = 25 minutes. At t = 0, the -CH_2 proton resonance (-CH_2-O_2C) in EtOAc, centered near 4.1 ppm, is nearly completely absent by t = 25 minutes. Even though the first spectrum is labeled t = 0 minutes, approximately 5 minutes had elapsed since the addition of acid before NMR analysis of this extracted sample was completed and, thus, it was

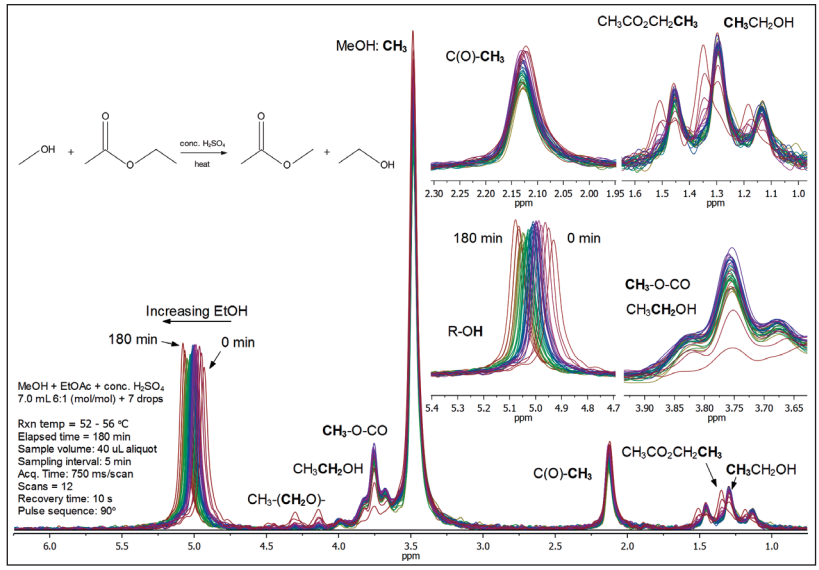

Figure 3: NMR spectra of the reaction mixture sampled at 5 minute intervals

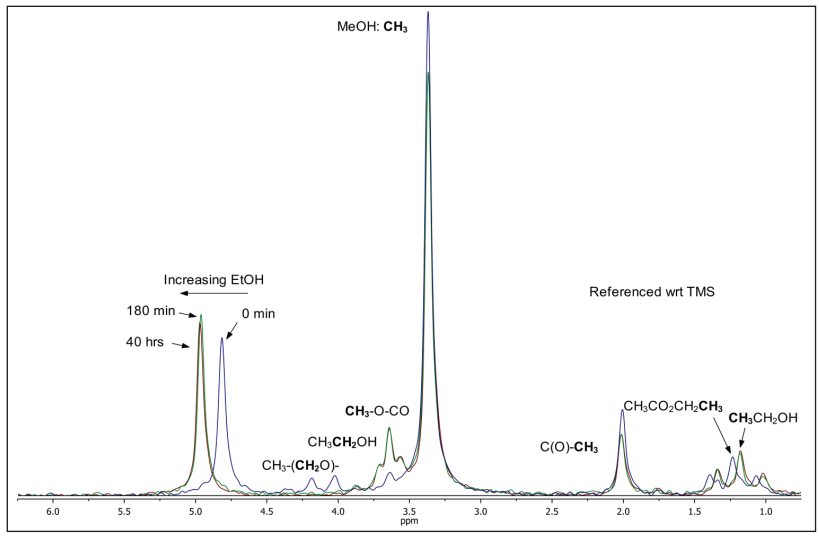

Figure 4: NMR spectra of the reaction mixture in MeOH measured at t = 0 minute, 180 minutes and 40 hours

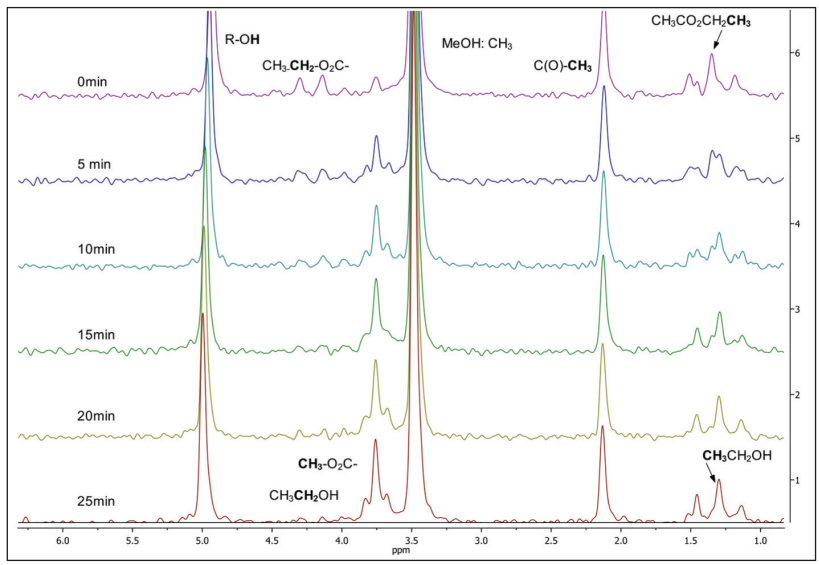

Figure 5: NMR spectra measured during the first 25 minutes of reaction at 5 minute intervals

not surprising that the changes in the initial reaction mixture had already occurred. This is most apparent in the weak signal emerging at 3.76 ppm which belongs to -CH_3 ester protons of the MeOAc product. As the reaction progresses, this signal continues to grow and -CH_2 signal from EtOH, which overlaps this -CH_3 ester signal, also begins to appear and grow. Further evidence of the extent of reaction can be seen in the triplet signal at 1.20 ppm where two overlapping triplets, one from the reactant EtOAc and the other from the product EtOH, are apparent already at t = 0 and coalesce into one triplet signal by t = 25 minutes.

The continual downfield shift of -OH peak up to t = 180 minutes and the comparison of this last spectrum to the one taken after 40 hours at first suggests the reaction is still progressing even at an elapsed reaction time of 3 hours. However, this behavior can be partially explained by evaporation. The position of -OH peak is sensitive to the mole fraction of alcohols in solution and as the reaction vessel was only loosely capped and the reaction temperature was kept close to the boiling point of MeOH (65 °C), the MeOH:EtOH mole ratio would favor EtOH as MeOH evaporates, shifting the -OH signal downfield with decreasing MeOH mole fraction.

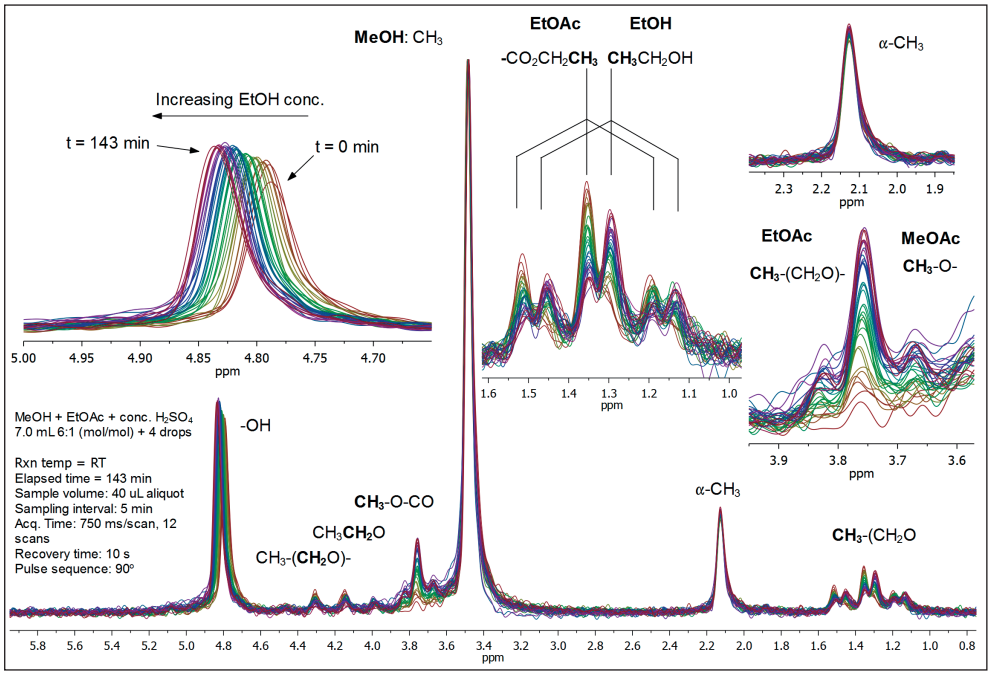

Figure 6: NMR spectra of the reaction mixture sampled at 5 minute intervals from a reaction conducted at room temperature and reduced amount of catalytic acid added

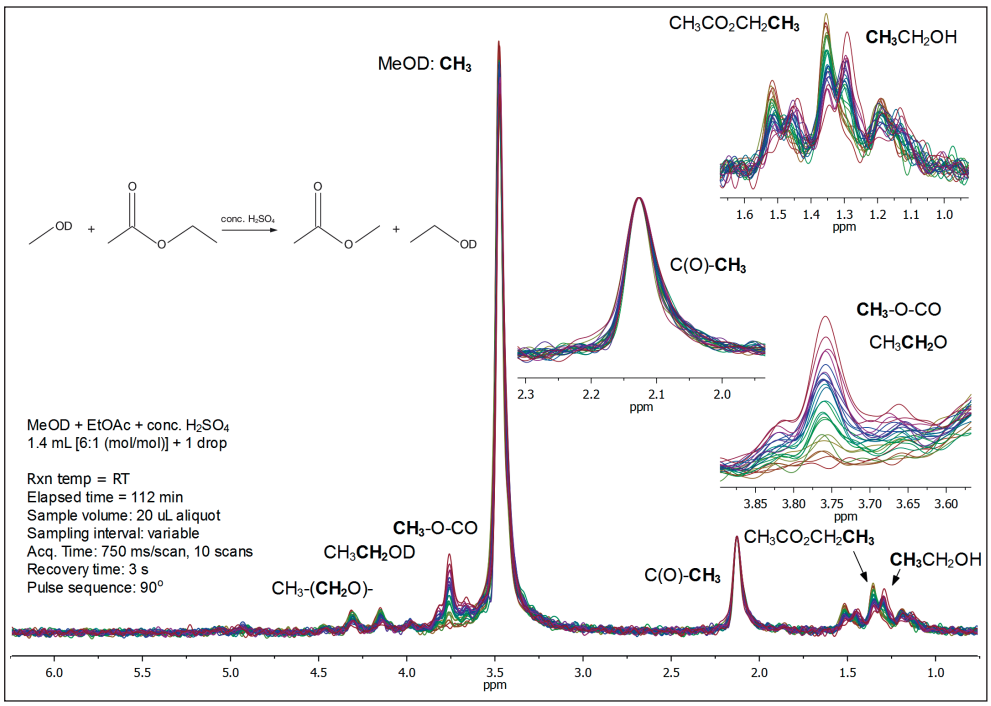

Figure 7: NMR spectra of the reaction mixture MeOD and EtOAc sampled at 3 minute intervals from a reaction conducted at room temperature and reduced amount of catalytic acid added

Slowing Down a Reaction

To approximate more closely a real-time analysis of the composition of the system during reaction, the reaction conditions were adjusted in order to slow down the reaction. By slowing down the reaction, we can observe chemical transformations occurring on the time-scale of NMR experiment. The spectra in Figures 3, 4 and 5 were catalyzed by concentrated H_2SO_4 and conducted at 52–56 °C.

In Figures 6 and 7, we made several modifications, such as:

- Run the reaction at room temperature,
- Reduce the amount of catalytic acid added,
- Shorten T1 recovery delay and the number of scans per sample, or
- Increase the sampling rate by shortening the time delay between sample injections.

Spectra in Figure 6 were obtained from a reaction mixture held at room temperature and with a reduced quantity of catalytic acid added. Individual spectra were normalized with respect to the peak maximum of the acetyl signal (2.13 ppm). This choice was justified since the keto-CH_3 group does not undergo chemical transformation during the reaction and, therefore, its signal intensity should remain constant throughout the experiment.

As the reaction proceeds, there is a downfield shift in the -OH peak position as the EtOH product concentration increases. Since the reactant MeOH is in large excess, its concentration is effectively constant during the reaction. Focusing on the -OH signal, the signal intensity is a minimum at t = 0 and increases in intensity as EtOH is produced. Clearly resolved is the transformation of the $-CH_3$ triplet signal from the reactant EtOAc into a $-CH_3$ triplet signal of the product EtOH as the reaction proceeds. The $-CH_3$ signal in EtOAc (1.35 ppm) is shifted 0.05 ppm downfield relative to the $-CH_3$ signal in EtOH (1.30 ppm) and diminishes more slowly under these reaction conditions, making its transformation easier to follow. Similarly, the $-CH_3$ signal (3.77 ppm) of the product MeOAc appears to still be growing even as the last spectrum is acquired.

In Figure 7, the reaction conditions were modified by substituting methanol-D (MeOD) for MeOH, running the reaction at room temperature and further reducing the quantity of catalytic acid added to the reaction mixture. Like Figure 6, spectra in Figure 7 are normalized relative to the peak maximum of the acetyl signal (2.13 ppm).

The most notable difference between the composite spectra in Figures 3, 6 and 7 is the absence of an -OH signal near 4.80 ppm due to the use of MeOD as a reactant in place of MeOH. As such, neither the reactant MeOH and product EtOH hydroxyl signals are observed. Otherwise, spectra in Figure 8 look similar except for the slower progression in growth of the $-CH_3$ ester signal at 3.77 ppm and the change in $-CH_3$ group signals at 1.30 and 1.35 ppm.

The growth of the $-CH_3$ ester signal as the reaction proceeds is clearly observed in Figure 8, where initial (t = 0 minute), intermediate (t = 25 minutes) and final (t = 112 minutes) spectra of the reaction mixture are presented. In Figure 9, the progression of the $-CH_3$ signal in the reactant EtOAc and the product ethanol-D (EtOD) is tracked.

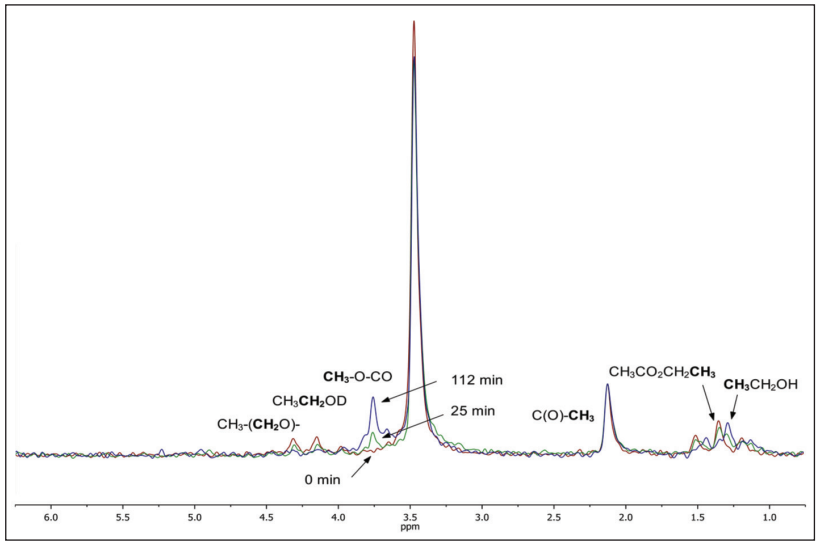

Figure 8: NMR spectra of the reaction mixture measured at t = 0 minute, 25 minutes and 112 minutes

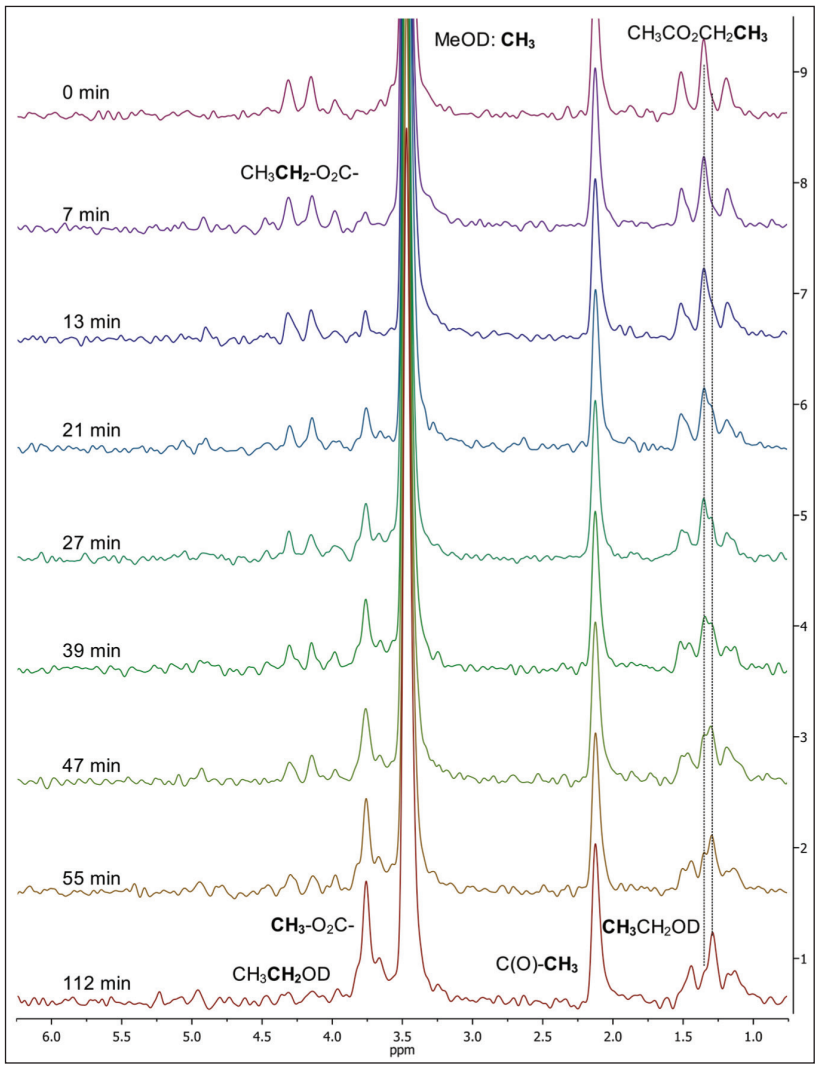

Figure 9: NMR spectra measured at various times during the course of the reaction

In Situ Monitoring

As a final test, we followed the course of reaction from a single injection of the reaction mixture into the RF coil region of the spectrometer's capillary probe. Figure 10 presents spectra measured at elapsed times of 9, 19, 28 and 41 minutes. Individual, single pulse spectra were measured every 5 seconds for 41 minutes. The spectra are normalized relative to the peak maximum of the acetyl signal.

These spectra show a similar conversion of EtOAc to MeOAc as in Figures 5 and 9; but in this case, we eliminated the possibility of MeOH evaporation which can account for changes in both the -CH_3 and the -OH signal intensity. For example, as the MeOH:EtOH mole fraction changes so will the chemical shift of the -OH signal, and reducing or eliminating evaporation of the lower boiling MeOH will allow for higher precision in chemometric analysis.

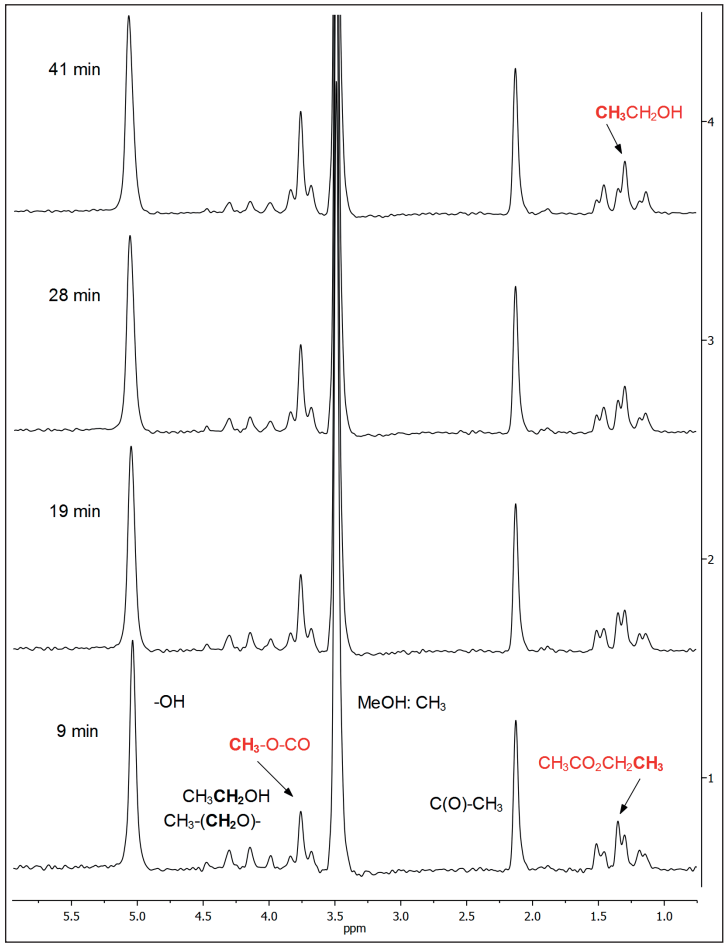

Figure 10: NMR averaged spectra of reaction mixture measured *in situ* at elapsed time of 9, 19, 28 and 41 minutes

Conclusion

The value of NMR as an analytical process tool in reaction monitoring is in its ability to provide qualitative and quantitative data without altering the underlying chemistry of the reaction. In this transesterification reaction, several ^1H signals in the NMR spectrum of the initial reaction mixture were used as markers to chart the evolution of the reaction. Even under different reaction and sampling conditions, progress in the reaction was readily observable. Through detailed analysis of this simple reaction, we have demonstrated the capabilities of the picoSpin 45 compact NMR spectrometer in reaction monitoring applications.

Abbreviations

µL = Microliter	**Hz** = Hertz
CH_2 = Methylene	**MeOAc** = Methyl Acetate
CH_3 = Methyl	**MeOD** = Methanol-D
EtOAc = Ethyl Acetate	**MeOH** = Methanol
EtOD = Ethanol-D	**MHz** = Megahertz
EtOH = Ethanol	**NMR** =Nuclear Magnetic Resonance
^1H = Proton	**OH** = Hydroxyl
H_2SO_4 = Sulfuric Acid	**PAT** = Process Analytical Technology
HDPE = High-density Polyethylene	**PPM** = Parts Per Million

References

1. Thermo Fisher Scientific, http://www.thermoscientific.com/picospin
2. Mestrelab Research, http://www.mestrelab.com

www.thermoscientific.com

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