Kinetic and Mechanistic Studies of the Deuterium Exchange in Classical Keto-Enol Tautomeric Equilibrium Reactions
Lab Documentation

Michael A. Nichols and Mark J. Waner*
Department of Chemistry, John Carroll University, 20700 North Park Blvd. University Heights, OH 44118.

Student Handout

Introduction:

In the first semester lab course, you may have determined the temperature dependence of the keto-enol equilibrium constants of acetylacetone (2, 4-pentanedione) and ethyl acetoacetate (ethyl 3-oxobutanoate) in chloroform-d_1 ($CDCl_3$) and methanol-d_4 (CD_3OD) using ^{1}H-NMR.[1-4] (See Figure 1.) Note that the two enol tautomers for acetylacetone rapidly equilibrate. We will use an "average" structure (in the box) to represent these two structures.

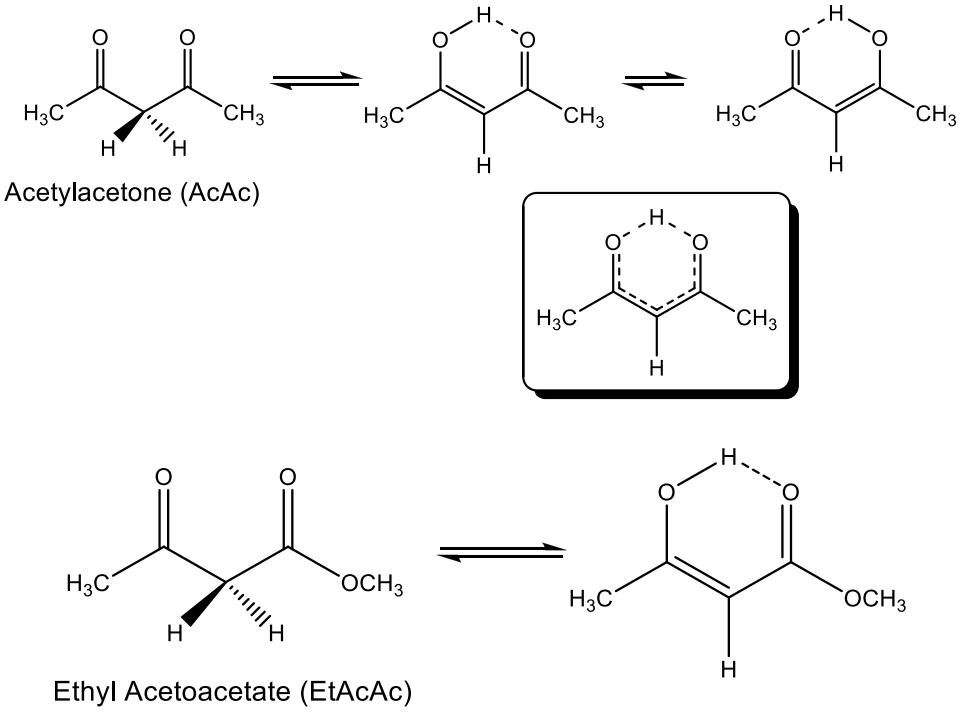


Figure 1. Keto-Enol Equilibria for Acetylacetone and Ethyl Acetoacetate.

[1] Adapted from Garland, C.W.; Nibler, J.W.; Shoemaker, D.P. *Experiments in Physical* Chemistry, 8th Ed. (and earlier editions) McGraw-Hill: New York, 2008; 466-474.
[2] Drexler, E.J.; Field, K.W. *J. Chem. Educ.* **1976**, *53*, 392-393.
[3] Grushow, A.; Zielinski, T.J. *J. Chem. Educ.* **2002**, *79*, 707-714.
[4] Cook, G.; Feltman, P.M. *J. Chem. Educ.* **2007**, *84*, 1827-1829.

When CD_3OD is used as the solvent for either β-dicarbonyl, an unpredicted 1:1:1 triplet (Figure 2) is observed at δ 3.50-3.65 in the NMR spectrum. The singlet at δ 3.52-3.67 corresponds to the methylene (CH_2) group of the β-diketone or β-ketoester, while the 1:1:1 triplet corresponds to the β-diketone or β-ketoester where one of the methylene hydrogen atoms has been replaced with a deuterium atom. To explain the 1:1:1 triplet pattern, we first need to recall (from organic spectroscopy) that the number of peaks in a proton coupling pattern is predicted by the equation n+1, where n is the number of adjacent protons. The general equation used to predict coupling patterns is $2n\boldsymbol{I}+1$, where n is the total number of coupling nuclei and $\boldsymbol{I}$ is the spin of that nucleus. Coupling to a proton, which has $\boldsymbol{I} = ½$, reduces the equation to the familiar n+1. However, in the monodeuterated dicarbonyl compound, geminal coupling occurs between the proton and deuterium nuclei. Since $\boldsymbol{I}$ = 1 for deuterium, the coupling pattern for the proton will be a triplet ($2 \cdot 1 \cdot \mathbf{1} + 1 = 3$).[5]

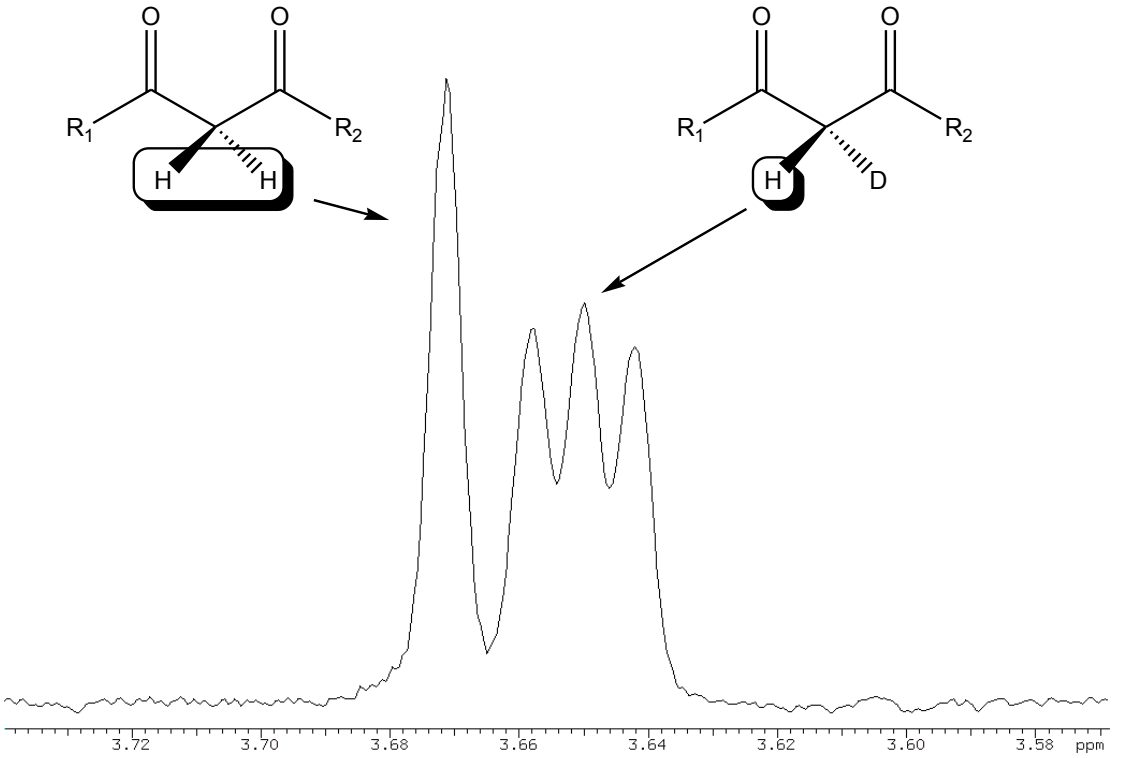


Figure 2. The Effect of Deuterium Substitution on the ^{1}H-NMR Spectrum of the α–Methylene Position of β-Diketones or β-Ketoesters.

In this experiment, you will be studying the kinetics of deuterium exchange between CD_3OD and each of the β-dicarbonyl compounds at 25°C using ^{1}H-NMR. The

[5] In organic spectroscopy, you learned that the intensities of the coupled peaks are governed by Pascal's Triangle. However, this is only true for nuclei with $\boldsymbol{I} = ½$. For nuclei with $\boldsymbol{I} = 1$, 1:1:1 triplets are predicted (see Jacobsen, N.E. *NMR Spectroscopy Explained: Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology*, Wiley-Interscience: New York, 2007, 131).

deuterium exchange reaction between ethanol-d_1 (CH_3CH_2OD) and ethyl acetoacetate was first reported in 1937[6] and has been measured in pure ethanol-d_1 and in basic ethanolic solutions by gas chromatography–mass spectrometry (GC-MS).[7] We can view the deuterium exchange between the β-dicarbonyl compound and CD_3OD as occurring by two consecutive reactions, shown in Figure 3. Both are truly equilibrium reactions, however, with CD_3OD as the solvent and in large excess, we will assume both are effectively irreversible and go to completion.

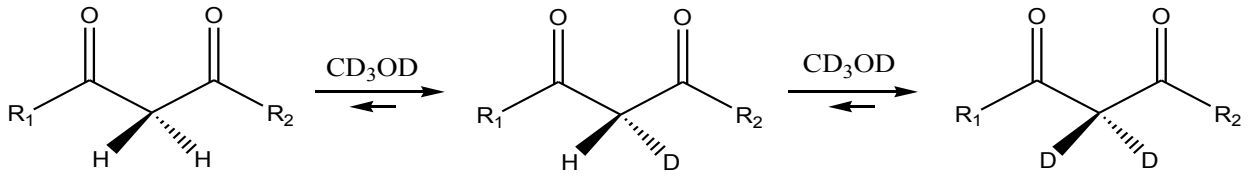


Figure 3. Deuterium Exchange Reactions Between CD_3OD and a β-Dicarbonyl Compound.

Including keto-enol equilibria into this process yields the proposed mechanism shown in Figure 4.[8] This mechanism involves four equilibrium steps and two essentially irreversible steps that (due to excess CD_3OD) drive the overall process to completion.

[6] Kharasch, M.S.; Brown, W.G.; McNab, J. *J. Org. Chem.* **1937**, *2*, 36-48.
[7] Heinson, C.D.; Williams, J.M.; Tinnerman, W.N.; Malloy, T.B. *J. Chem. Educ.* **2005**, *82*, 787-789.
[8] The mechanism for AcAc is shown; the mechanism for EtAcAc is nearly identical.

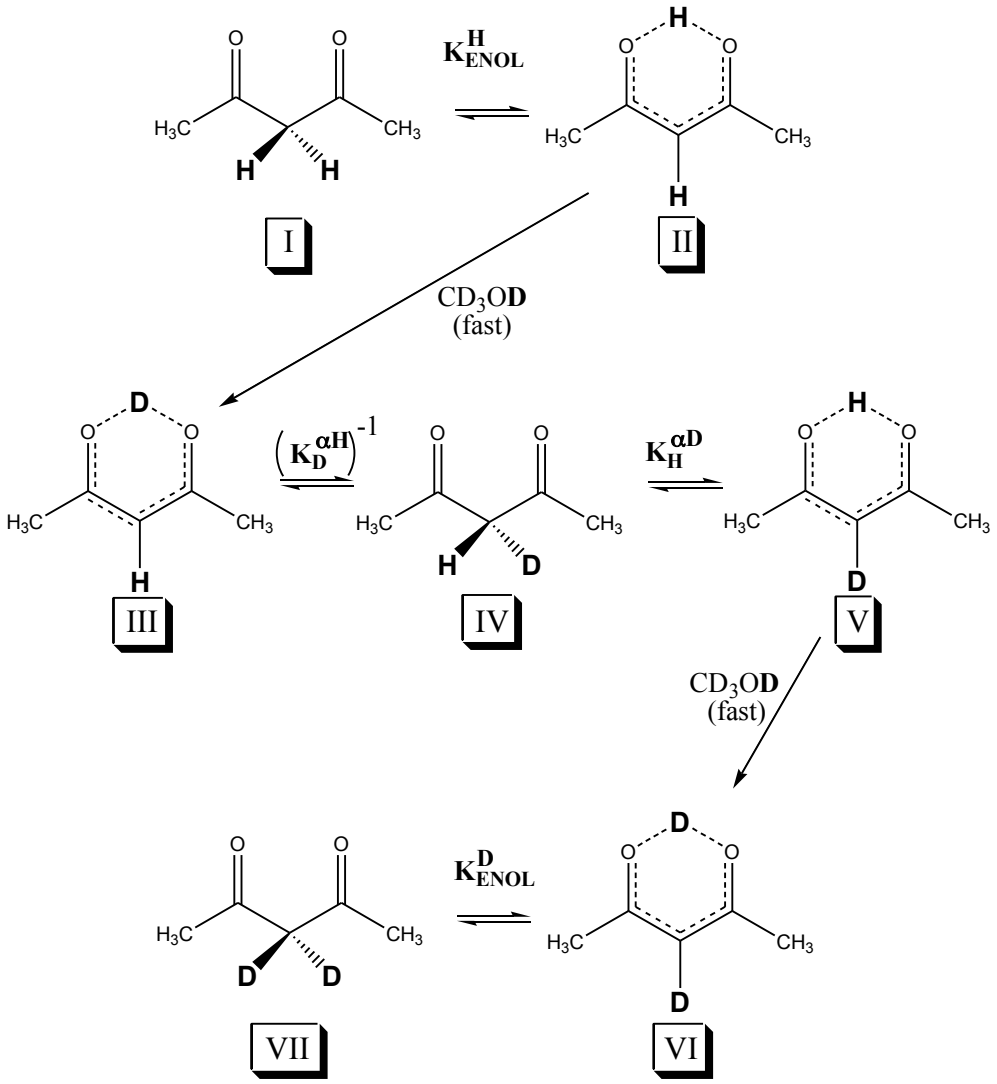


Figure 4. A Proposed Mechanism of Deuterium Exchange Between CD_3OD and Acetylacetone.

K^{H}_{ENOL} can be determined by the standard NMR experiment[1-4] using CD_3OH as the solvent, since it has no exchangeable D. K^{D}_{ENOL} can be determined in the same way after allowing the overall exchange reaction to go to near completion (~72 hours). Martin and coworkers[9] have used site-specific natural isotope fractionation by NMR (SNIF-NMR) to determine $K^{\alpha H}_{D}$ and $K^{\alpha D}_{H}$ in neat acetylacetone and ethyl acetoacetate.

In the proposed deuterium exchange mechanism, seven distinct species are part of the consecutive equilibrium process. (See Figure 4.) By recording individual ^{1}H-NMR spectra as a function of time, the overall process can be monitored and the concentrations of most species can be directly or indirectly quantified. A calibrated

[9] Zhang, B.L., Mabon, F., Martin, M.L. *J. Phys. Org. Chem.* **1993**, *6*, 367-373.

sealed internal capillary containing benzene and $CDCl_3$ will be placed in the NMR tube to allow the NMR resonances to be normalized and the integrations converted to concentrations.

The concentrations of the ketone-CH_2 (**I**, singlet methylene peak), enol-CH (sum of **II** and **III**, singlet vinylic H peak), and ketone-CHD (**IV**, 1:1:1 triplet peak) can be determined directly from NMR integrations of the indicated resonances. The concentrations of the enol-CD (sum of **V** and **VI**) and enol-CD_2 (**VII**) species must be determined indirectly by difference. The concentration of the enol-CD species (sum of **V** and **VI**) will be the difference between the total concentration of all enol species (**II**, **III**, **V** and **VI**, determined from the integration of the enol CH_3 singlet resonance) and the enol-CH species. The concentration of the ketone-CD_2 species (**VII**) can be calculated by subtracting the concentrations of the ketone-CH_2 (**I**) and ketone-CHD (**IV**) species from the total concentration of all ketone species (**I**, **IV**, and **VII**, determined from the integration of the ketone CH_3 singlet resonance).

A full kinetic analysis of the consecutive equilibrium process of the deuterium exchange between acetylacetone and ethyl acetoacetate in CD_3OD (Figure 4) is complex (i.e. the system of differential equations does not have an analytic solution). To simplify the analysis, the concentrations of several intermediate species will be combined and the kinetic data will be modeled as a first order consecutive reaction.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

In this model, **B** behaves as an intermediate. The integrated rate laws for each species may be determined from the rate expressions and mass action principles.[10]

$$[A] = [A]_o e^{-k_1 t} \tag{1}$$

$$[B] = \frac{k_1[A]_o}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \tag{2}$$

$$[C] = [A]_o\left[1 - \frac{k_2}{k_2 - k_1}e^{-k_1 t} + \frac{k_1}{k_2 - k_1}e^{-k_2 t}\right] \tag{3}$$

with $[B]_o = [C]_o = 0$

The simplified reaction sequence shown in Figure 3, fits the A → B → C model. Combining this simple model with the proposed mechanism in Figure 4, leads to the scheme shown in Figure 5. In this simplified scheme, **B** (the monodeuterated ketone, **IV**) is the intermediate species. One of the analyses you will perform is to determine whether it behaves according to steady state kinetics.

[10] Steinfeld, J.I.; Francisco, J.S.; Hase, W.L. *Chemical Kinetics and Dynamics,* 2nd Ed. Prentice-Hall: Upper Saddle River, NJ, 1999, 26-27.

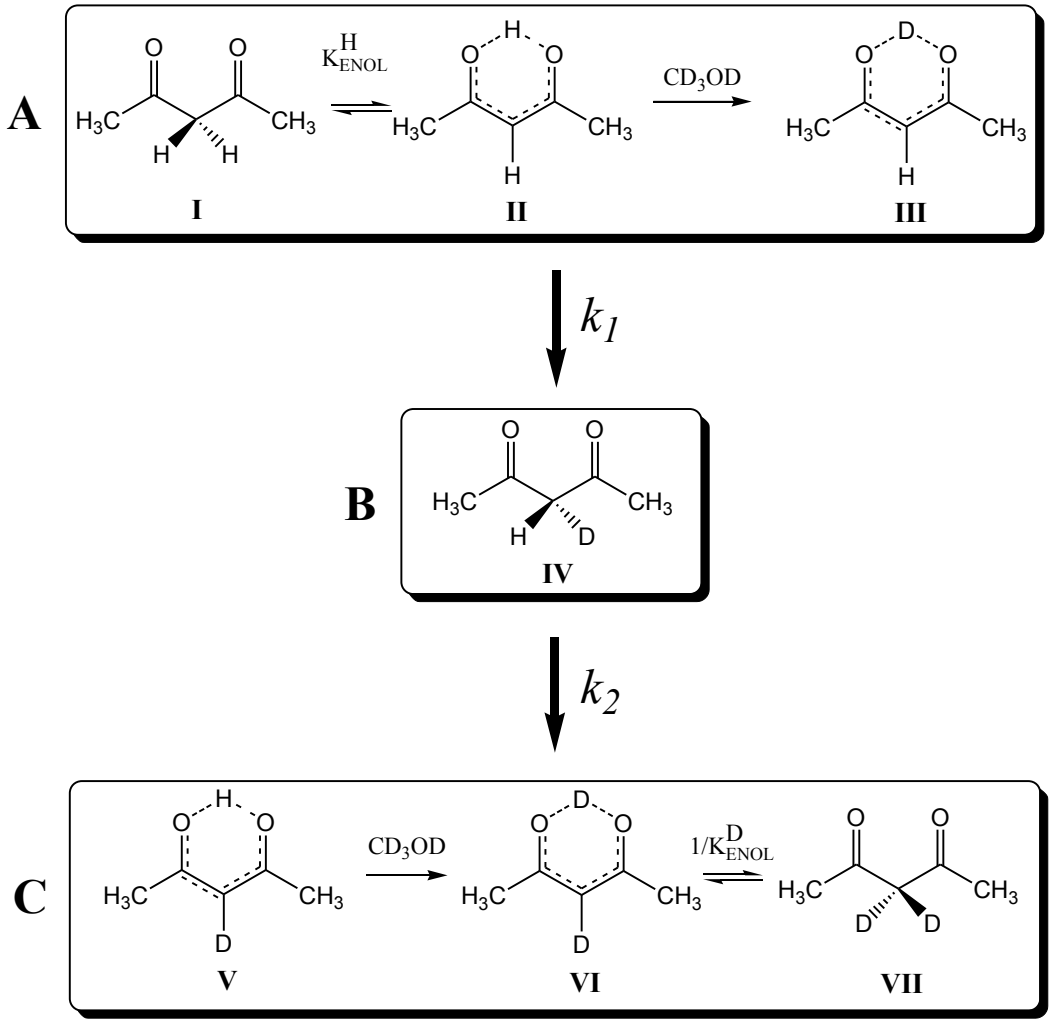


Figure 5. A Simplified Reaction Scheme for the Deuterium Exchange Between Acetylacetone and CD_3OD.

Materials:

Chemicals.

- **Acetoacetone** (2,4-Pentanedione, CAS No. 123-54-6, Fluka, > 99.5% puriss.)
- **Ethyl Acetoacetate** (Ethyl 3-oxobutanoate, CAS No. 141-97-9, Sigma-Aldrich, 99% purity)
- **Methanol-*d*$_4$** (CAS No. 811-98-3, Sigma-Aldrich, 99.8%D)
- **Methanol-*d*$_3$** (CAS No. 1849-29-2, Sigma-Aldrich, 99.8%D)
- **Benzene** (CAS No. 71-43-2, Fisher Scientific, ACS Certified Grade, Thiophene Free, stored with 4Å molecular sieves or purified by distillation)
- **Chloroform-*d*** (CAS No. 865-49-6, Sigma-Aldrich, 99.8%D w/ 0.03% v/v TMS)

Equipment and Instrumentation.

- 5 mm NMR Tubes, Norell® Standard Series, ST500-7 or equivalent
- 5 mm Rubber Septa, Norell® SEPTA-5-W or equivalent
- 1 mL Adjustable Micropipettor and Tips
- Melting Point Capillary Tubes
- Micro-torch
- 25 μL Gas-Tight GC Syringes
- 100 μL GC Syringe
- Pipe Cleaner or small piece of wire to remove the capillary from the NMR tube
- Varian 300 MHz NMR System with VnmrJ software (v. 2.1b), and equipped with PFG, broadband, and variable temperature accessories.

Safety Information.

- ***Wear eye protection at all times and hand protection when preparing the internal capillary and NMR tubes.***
- ***Prepare the internal capillary and NMR tubes in a hood.***
- Material Safety Data Sheets (MSDS) for each of the chemicals used in the experiment are available at http://www.sigmaaldrich.com and should be consulted prior to conducting the experiment.
- **Acetoacetate:** Flammable; Toxic if swallowed; Irritating to eyes, respiratory system and skin; Neurological hazard; Target organs: thymus, nerves.
- **Ethyl Acetylacetate:** Irritant; Irritating to eyes; Combustible liquid.
- **Methanol-*d*$_4$, Methanol-*d*$_3$:** Flammable; Toxic by inhalation, in contact with skin, and if swallowed; Danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed; May be fatal or cause blindness if swallowed. Irritating to eyes and skin; Target organs: eyes, kidneys.
- **Benzene:** Flammable liquid; Irritant; Carcinogen; Mutagen; Target organs: Blood, eyes, female reproductive system, bone marrow.
- **Chloroform-*d*:** Irritating to eyes and skin; Harmful if swallowed; Danger of serious damage to health by prolonged exposure through inhalation and if

swallowed; Probable carcinogen; Probable mutagen; Target organs: liver, cardiovascular system.

Procedure:

Measurement of K_{ENOL}^{H}. The keto-enol equilibrium constant K_{ENOL}^{H} cannot be accurately measured in CD_3OD due to the deuterium exchange reaction; it must be measured in methanol-d_3 (CD_3OH). To measure K_{ENOL}^{H}, turn on and set the variable temperature to 25°C. Add 0.75 to 1 mL of CD_3OH to a clean NMR tube using a micropipette and cap it with a rubber septum. Place the sample into the NMR spinner and lower it into the NMR magnet. Lock and shim the sample and acquire a ^{1}H-NMR spectrum using quantitative parameters:

- Pulse width (PW) of 5 µs.
- Delay time (D1) of 60 s.
- Number of scans (NT) equal to 4.

A large, broad peak should be observed at approximately δ 4.8 ppm (-OH) and a small pentuplet at δ 3.3 ppm (CD_3). If the peaks in the spectrum show symmetrical line shape and no impurity peaks are observed, then eject the sample and add 20 µL acetylacetone to the NMR tube. Shake well, place the sample back into the magnet. Allow the sample to equilibrate at 25°C for 10 minutes. Then lock, shim, and acquire a proton spectrum using the same parameters. Integrate the enol-CH, ketone-CH_2, enol-CH_3 and ketone-CH_3 singlet peaks. Calculate K_{ENOL}^{H} independently from the enol-CH : ketone-CH_2 and enol-CH_3 : ketone-CH_3 integration ratios. Report the average and use this value to determine the percentages of enol and ketone and ΔG_{ENOL}. Repeat this procedure for ethyl acetoacetate.

Preparation of the Capillary Internal Standard. Add 25 µL of purified benzene to approximately 0.5 mL of $CDCl_3$. Mix thoroughly. Fill several melting point capillary tubes approximately to ~80% capacity with this solution using a 100 µL GC syringe. Seal the melting point capillary tube with a micro-torch.

Calibration of the Capillary Internal Standard. Obtain a clean 5 mm NMR tube and add 0.75 mL of $CDCl_3$ via micropipette. Wipe off all fingerprints from the capillary tube and carefully place it into the NMR tube and cap it with a rubber septum. Wipe off all fingerprints from the NMR tube and place it in a small beaker. Place the beaker/NMR tube on an analytical balance and tare it. Using a GC syringe, add 15 µL of purified tetrahydrofuran (THF). Wipe any fingerprints from the NMR tube and reweigh it. The mass of THF added should be 13-14 mg. Re-tare the NMR tube/beaker. Using a second GC syringe, add 20 µL of dichloromethane and determine the mass of dichloromethane added (should be approximately 30 mg).

Invert the NMR tube several times to mix the solution. Acquire a ^{1}H-NMR spectrum using the following parameters:

Sweep Width (SW, Hz)	4808	Acquisition Time (AT, s)	3.5	Number of Points (NP)	33,654
Number of Scans (NT)	1	Spectrometer Frequency (SFRQ, MHz)	300.07	Pulse Width (PW μs)	5
Delay (D1, s)	1	Transmitter Gain (GAIN, dB)	30	Line Broadening (LB, Hz)	0.2

Phase the spectrum and integrate the dichloromethane, benzene internal standard and two THF resonances.

Calculation of the Capillary Tube Calibration Constant. From the measured masses of THF and CH_2Cl_2 determine the total number of protons (in moles) for each analyte. Then, determine the number of protons per integration unit for each (remember to take the sum of integrations for both THF peaks). Using these two values determine the average number of protons per integration unit and multiply this by the measured integration of the benzene peak. You should get the number of moles of protons contained in the benzene/$CDCl_3$ capillary.

Performing the Arrayed Kinetic Experiment. Turn on the variable temperature unit of the NMR, set the probe temperature at 25°C, and allow the instrument to equilibrate for at least 10 minutes. Remove your newly calibrated sealed internal capillary using a pipe cleaner and then carefully wipe it with a Kimwipe. Place it into a clean NMR tube and add 0.75 to 1 mL of CD_3OD to the tube using a micropipette. Cap the NMR tube with a rubber septum. Make sure that the internal capillary is resting at the bottom of the NMR tube and place it in the magnet. Allow your sample to equilibrate at 25°C for approximately 10 min., and lock, shim, and acquire a ^{1}H-NMR spectrum of your solvent to make sure that the sample tube is well shimmed (well resolved methanol-d_4 pentuplet at δ 3.30 ppm) and there are no apparent contaminants. Use the same parameters you used in the calibration of the capillary internal standard (above).

Obtain a 25 μL GC syringe and fill with 20 μL of acetylacetone. Wipe off any excess liquid and all fingerprints from the syringe and determine its mass. Eject the sample and add the 20 μL of acetylacetone. Quickly invert the NMR tube several times to mix. Start the stopwatch as soon as you invert the tube the first time. Wipe any fingerprints from the NMR tube, place the tube back into the spinner assembly, adjusting the tube to the proper depth, and insert the spinner/tube back into the magnet. Make sure that the sample spins at the proper rate. When the stopwatch reaches 1 minute, start the kinetic array data collection. Wipe off any fingerprints from the GC

syringe and reweigh it. The mass difference will be the amount of acetylacetone added to the NMR tube. You can use this value to calculate the concentration of the dicarbonyl compound. In the experiment, an NMR spectrum will be taken after starting the array (at 2 min), then 81 spectra will be collected at 2 min intervals and finally an additional 41 spectra will be collected at 4 min intervals. A total of 123 spectra will be collected in the array over a 327 minute total reaction time. After the data collection has finished, you should **save the data file**, before starting any analyses.

Measurement of K^{D}_{ENOL}. Deuteration of the β-dicarbonyl compound is nearly complete after 72 hr. After performing each kinetic array NMR experiment, label and set aside the NMR tube.[11] After 72 hr, collect an NMR spectrum and follow the procedure you used to determine K^{H}_{ENOL}. Integrate the total enol CH_3 and ketone-CH_3 singlets, calculate K^{D}_{ENOL}, and determine the percentages of enol and ketone and ΔG_{ENOL}. Perform this procedure for both acetylacetone and ethyl acetoacetate.

Kinetic Data Processing. The NMR array file contains all 123 spectra. You will need to process and integrate each NMR spectrum and then create a data table of the integration areas for each resonance. In the VnmrJ software, you move between spectra by typing the command **ds(*x*)**, where *x* is the number of the spectrum in the array. The most efficient way to integrate each spectrum is to first choose a spectrum that has all the species present. For example, Figure 6 shows the 5th spectrum in the array (time = 9 min) for the reaction of acetylacetone with CD_3OD at 25°C.

[11] The internal sealed capillary tube can be removed from the NMR tube using a pipe cleaner; be sure to carefully wipe it thoroughly before placing it into the next NMR sample.

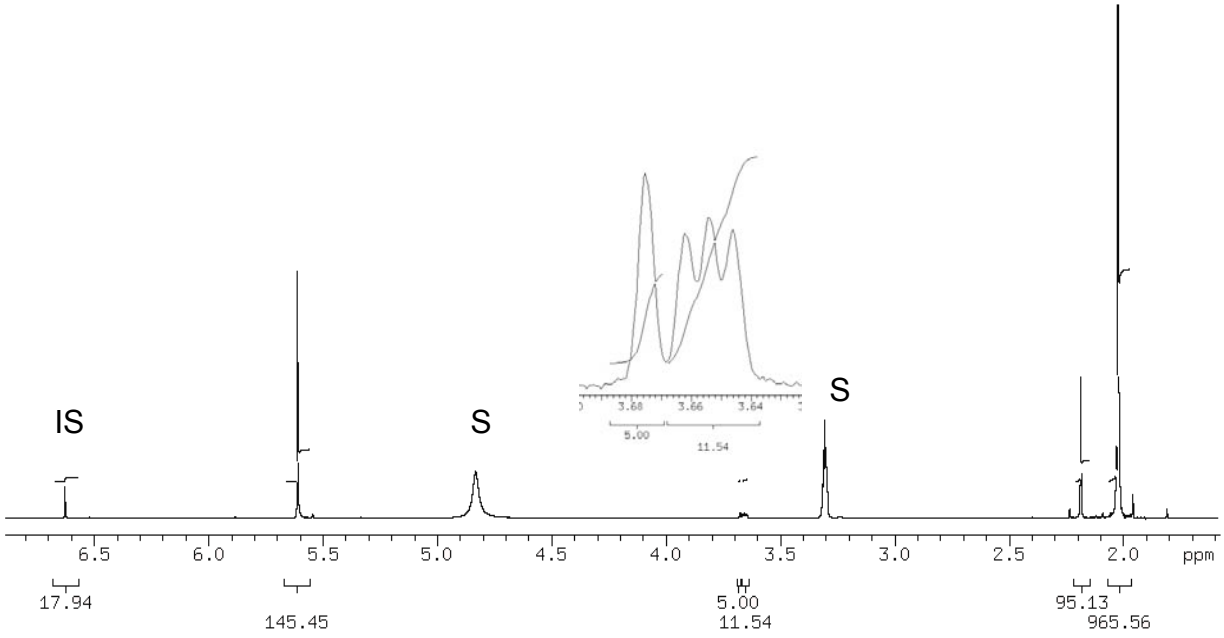


Figure 6. An Example NMR Spectrum from the Reaction of Acetylacetone and CD_3OD at 25°C. The reaction time was 8 minutes. The inset shows the singlet and 1:1:1 triplet of species **I** and **IV**, respectively. The resonances corresponding to the benzene internal standard (IS) and of the CD_3OD solvent (S) are indicated.

By defining the integral regions using a spectrum that has all of the species visible, you should not have to set them again for the spectra in the array. Starting with the first arrayed spectrum, you will: (1) display it (**ds(*x*)**); (2) phase it (use autophasing, **aph**); (3) display the integral region markers (click the green integral button in the **Spectrum Display Buttons**); and (4) display the numerical integrals on the screen by selecting the **Process** parameter tab, then the **Cursors/Integration** parameter page, entering a **Normalization Value** of 100 and then clicking on the **Normalized Values** box. You should then enter these integral values into your spreadsheet. Repeat the process for each spectrum in the array.[12]

Normalization of the Integration Values. In theory, the integration value obtained for the internal standard should be exactly the same in each NMR spectrum. However, due to instrumental and analysis uncertainties, the internal standard integrations will vary slightly over the course of the experiment. The second step in data processing is to normalize the data to correct for these variations. A sample set of data will be used throughout the next few processing steps. As a check of the consistency, you should verify that the internal standard integrations don't vary by more than 10%. If you find an outlier, go back and examine / reintegrate that NMR spectrum;

[12] You may need to manually phase and reintegrate some of the spectra if the integral markers are not completely horizontal (ie. tilt) or do not completely cover the peak(s).

be sure to check the phasing of the spectrum. Notice that in Table 1 there is only 2-3% variation in the integral values for the internal standard.

Table 1. A Sample Set of Raw Integration Data.

Raw Integration Data						
Spectrum (Array No.)	**Internal Standard**	**Enol CH (II + III)**	**Ketone CH_2 (I)**	**Ketone CHD (IV)**	**Total Ketone CH_3's (I + IV + VII)**	**Total Enol CH_3's (II + III+ V + VI)**
1	46.09	341.70	75.26	12.91	302.73	2079.34
2	48.65	356.49	44.93	18.34	249.25	2156.70
3	46.60	354.22	27.46	22.66	227.73	2194.76
Average	47.11					

The first step in the normalization is to determine the average of all internal standard integrations; all integration values will be normalized to this value using the equation below:

$$\text{Norm. Integ.} = \text{Raw Integ.} \times \frac{\text{Ave. Int. Std. Integr.}}{\text{Spectrum Int. Std. Integ.}}$$

The normalized data is shown in Table 2.

Conversion of Integral Values to Concentrations. The benzene/$CDCl_3$ internal capillary is used to normalize the integrations and to convert those values to concentrations.

Table 2. A Sample Set of Normalized Integration Data.

Normalized Integrations						
Spectrum (Array No.)	**Internal Standard**	**Enol CH (II + III)**	**Ketone CH_2 (I)**	**Ketone CHD (IV)**	**Total Ketone CH_3's (I + IV + VII)**	**Total Enol CH_3's (II + III+ V + VI)**
1	47.11	349.29	76.93	13.20	309.45	2125.51
2	47.11	345.23	43.51	17.76	241.38	2088.58
3	47.11	358.12	27.76	22.91	230.24	2218.94

To convert the normalized integration of a peak to the concentration of that species, use the formula below:

$$[\text{Species}](\text{M}) = \frac{\text{Integr. of Species Peak}}{0.00075\ \text{L}} \times \frac{\text{Moles of Species}}{\text{Moles of H in Species Peak}} \times \frac{\text{Moles of H in Int. Std.}}{\text{Normal. Integr.}}$$

Where: **Moles of H in Int. Std.** was determined from the calibration experiment
Normal. Integr. is the normalized integration for the internal standard
Integr. of Species Peak is the integration of the NMR peak of that species

Examples of the **moles of species** : **moles of H's in species peak** ratio include: for every 1 mole of enol-CH (**II** + **III**), there is 1 mole of H in that peak; for every 1 mole of ketone-CH_2 (species **I**), there are 2 moles of protons in that peak.
The volume in the NMR tube is 0.75 mL (0.00075 L).

The concentrations calculated from the integrations shown in Table 2 are shown in Table 3.

Table 3. A Sample Set of Concentration Data.

		Concentration (in M)				
Spectrum (Array No.)	**Internal Standard**	**Enol CH (II + III)**	**Ketone CH_2 (I)**	**Ketone CHD (IV)**	**Total Ketone CH_3's (I + IV + VII)**	**Total Enol CH_3's (II + III+ V + VI)**
1	47.11	0.219	0.024	0.008	0.032	0.223
2	47.11	0.217	0.014	0.011	0.025	0.219
3	47.11	0.225	0.009	0.014	0.024	0.232

Calculation of Enol-CD (V + VI) and Ketone-CD_2 (VII) Concentrations. The concentrations of **V**, **VI**, and **VII** cannot be determined directly from the ^{1}H-NMR integral values because of the deuterium substitution. They can, however, be determined indirectly by subtraction. **The key to this analysis is to recognize that the concentrations determined using the CH_3 resonances correspond to the <u>total enol and total ketone concentrations</u>.** The enol-CD species (**V** + **VI**) is calculated by subtracting the enol-CH (**II** + **III**) concentration from the total enol concentration. The ketone-CD_2 (**VII**) concentration is calculated by subtracting the ketone-CH_2 (**I**) and ketone-CHD (**IV**) concentrations from the total ketone concentrations. Table 4 shows the complete concentration data.

Table 4. A Sample Set of Complete Concentration Data.

		Conc. (M)						
Spectrum (Array No.)	**Int. Std.**	**Enol CH (II+III)**	**Ketone CH_2 (I)**	**Ketone CHD (IV)**	**Total Ketone CH_3's (I+IV+VII)**	**Total Enol CH_3's (II+III+V+VI)**	**Enol CD (V + VI)**	**Ketone CD_2 (VII)**
1	47.11	0.219	0.024	0.008	0.032	0.223	0.003	0.000
2	47.11	0.217	0.014	0.011	0.025	0.219	0.002	0.000
3	47.11	0.225	0.009	0.014	0.024	0.232	0.007	0.001

Results:

Comparison of NMR and Mass Calculated Concentrations. Calculate the concentrations of keto/enol from the mass of acetylacetone that was added to the total volume of 0.75 mL in the NMR tube. Calculate the total concentration of keto and enol forms of the β-dicarbonyl using the CH_3 protons for each spectrum in the NMR array and prepare a plot the total concentration vs. time and determine the average concentration and standard deviation. Calculate the percent error for the average concentration and the one calculated from the mass added to the NMR tube. Repeat this for the ethyl acetoacetate experiment.

Plots and Consecutive Reaction Modeling Results.

(a) Prepare a graph of the enol-CH (**II+III**), ketone-CH_2 (**I**) ketone-CHD (**IV**), enol-CD (**V+VI**) and ketone-CD_2 (**VII**) concentrations vs. reaction time.

(b) Using the scheme shown in Figure 5, calculate and plot the concentrations of A, B and C versus time.

(c) Verify that the initial reaction of species A (Figure 5) with CD_3OD is pseudo[13]-first order.

(d) Use the appropriate plot from part c above to get an estimate of the first kinetic rate constant (k_1) to use in the simplified first order consecutive reaction model.

(e) Determine the value of k_2 by fitting the [B] vs. time using Equation 1, 3.

(f) Using the values of k_1 and k_2 plot the predicted and measured [C] vs. time.

Some questions to consider as you write the discussion section of your report:

1. You measured K^{H}_{ENOL} and K^{D}_{ENOL} for acetylacetone and ethyl acetoacetate. Does complete α-methylene deuteration significantly affect the K_{ENOL} and ΔG_{ENOL} values?
2. At what time are the rates of the first deuterium exchange and the second equal?
3. What is the relative concentration of intermediate (B) to reactant species (A) at this point? Compare acetylacetone and ethyl acetoacetate, why are the values different for these two substances?
4. Does the steady state approximation hold for the deuterium exchange with either acetylacetone or ethyl acetoacetate? Describe why or why not in terms of your observed k_1 and k_2 values and the observed plots of species A, B and C.
5. Assume that the ΔG_{ENOL} for diethyl malonate is 8.6 kJ/mol. Would you expect this to behave similarly, with respect to deuterium exchange in CD_3OD, to acetylacetone or ethyl acetoacetate?

[13] The kinetics are most accurately described as pseudo-order as the CD_3OD is in large excess and therefore its concentration does not change significantly during the reaction (i.e. CD_3OD is pseudo-zero order).

Instructor Notes

The student handout we have given is rather prescriptive in terms of the introduction and procedural details provided. This was done to provide a complete set of experimental details to instructors unfamiliar with performing this type of kinetic NMR experiment. To be sure, in this manuscript we have added to the actual handout we used with our second semester physical chemistry laboratory students. In that case we knew the students' previous experiences with this keto-enol tautomerization, kinetics and the use of NMR instrument. In that case, we were more directive in the use of the internal calibration standard and normalization, but did not provide all the details for determining concentrations of the various species or the fitting of the data.

We also included instructions for measuring $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{H}}$ and $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{D}}$, and $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{H}}$ for the neat dicarbonyl compounds (see procedure outlined below), although they are not necessary for the kinetic analysis presented. If time permits, having students determine these equilibrium values will allow them to determine whether the overall deuterium exchange affects the equilibrium constants. Measuring $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{H}}$ for the neat compounds reinforces the differences in equilibrium position of acetylacetone and ethyl acetoacetate and demonstrates that although we most often write the structure of acetylacetone in its keto form, it is mostly enol in its pure form. As Cook and Feltman[4] point out, comparison of the $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{H}}$ values of the carbonyls in their neat and solvated forms aids the student in rationalizing solvent effects on the keto-enol tautomerizm.

We presume that instructors will want to tailor this handout to their own learning goals and the prior experience of their students. Given a longer period of time, such as a 3-4 week laboratory project, we could see the students being guided to the initial observation of the triplet, then allowing them to discover the explanation for it and to investigate the kinetics of the process.

Measuring K_{ENOL} for neat β-dicarbonyls:

The Garland, Shoemaker and Nibler physical chemistry laboratory text cites the ΔG_{ENOL} for gas phase acetylacetone and ethyl acetoacetate. Another useful comparison for this experiment, and the classical one, is to measure K_{ENOL} for neat β-dicarbonyls. We have done this by placing an open melting point capillary, ~80% filled with acetylacetone or ethyl acetoacetate into ~0.75 mL $CDCl_3$. These values compare well with those reported by Cook and Feltman.[4] (See Table 1 below.)

Measuring K_{ENOL} for β-dicarbonyls in methanol-d_3 and d_4:

We have measured the $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{H}}$ and $\mathbf{K}_{\mathrm{ENOL}}^{\mathbf{D}}$ values for acetylacetone and ethyl acetoacetate in CD_3OH and CD_3OD and there is no significant difference. (See Table 1.) However, a large shift is observed for neat vs. methanol solvated β-dicarbonyl. The K_{ENOL} values for neat acetylacetone and ethyl acetoacetate measured were approximately 8% higher than those given by Martin et al.[9] These values are consistent with 83% enol for acetylacetone and 8% enol for ethyl acetoacetate. The equilibrium constant decreases significantly when moving into a polar solvent; we find about 75% enol for acetylacetone and 6% enol for ethyl acetoacetate in methanol-d_3

and $-d_4$. The K_{ENOL} value measured for ethyl acetoacetate in methanol-d_3 and $-d_4$ is comparable to the 0.068 measured in CH_3OH by Ruggiero and Luaces.[14]

Acetylacetone	K_{ENOL}
Neat	4.81 (4.42)[9]
In CD_3OH	3.08
In CD_3OD	3.0
Ethyl Acetoacetate	K_{ENOL}
Neat	0.085 (0.079)[9]
In CD_3OH	0.060
In CD_3OD	0.063

Table 1: Equilibrium Constants for the Tautomerization of Acetylacetone and Ethyl Acetoacetate.

Collection of Kinetic Data at Elevated Temperature:

We have collected data at 40°C, which significantly cuts down the reaction time. However, the concentration vs. time data contained significantly more variability. This appears to be linked to incomplete sealing of the internal standard capillary, as we noted decreased intensity of the benzene in the internal standard as time progressed. With a properly sealed capillary one could cut down the data collection time to about 2.5 to 3 hours at 40°C.

Time can also be used more efficiently if the NMR system allows for parallel analysis during data collection.

Sealing the Capillary Internal Standard:

We have found that filling an ordinary melting point capillary tube to 75-80% capacity and then slightly touching to top of the tube with a focused micro-torch flame while rotating the tube in your fingers will seal the tube. Once sealed, the tube will form a small bulb; quickly remove the tube from the flame and cool by blowing on it.

[14] Ruggiero, S.J. and Luaces, V.M. *J. Chem. Educ.* **1988**, *65*, 629.

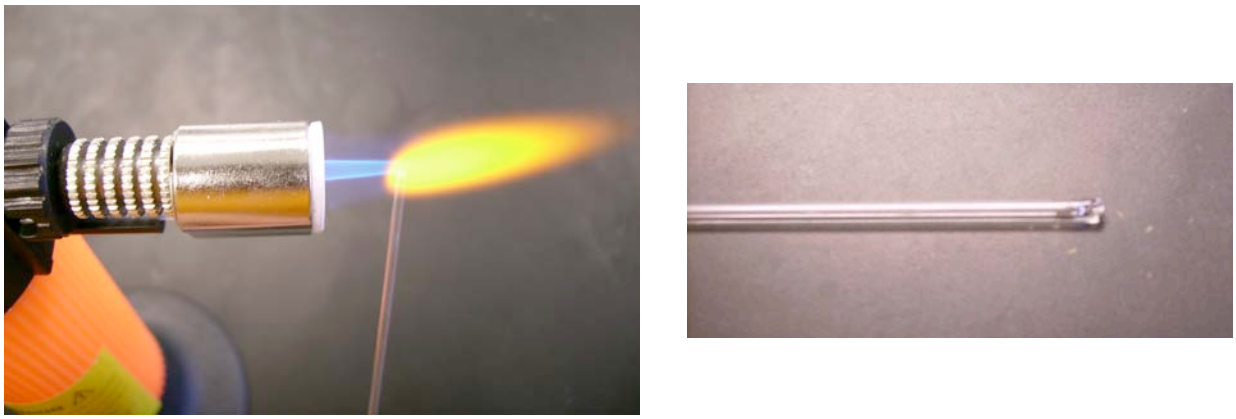


Figure 1. Flame Sealing the Capillary Internal Standard with a Micro-torch.

Calibration of the Capillary Internal Standard:

A sample NMR spectrum for a calibration is shown below:

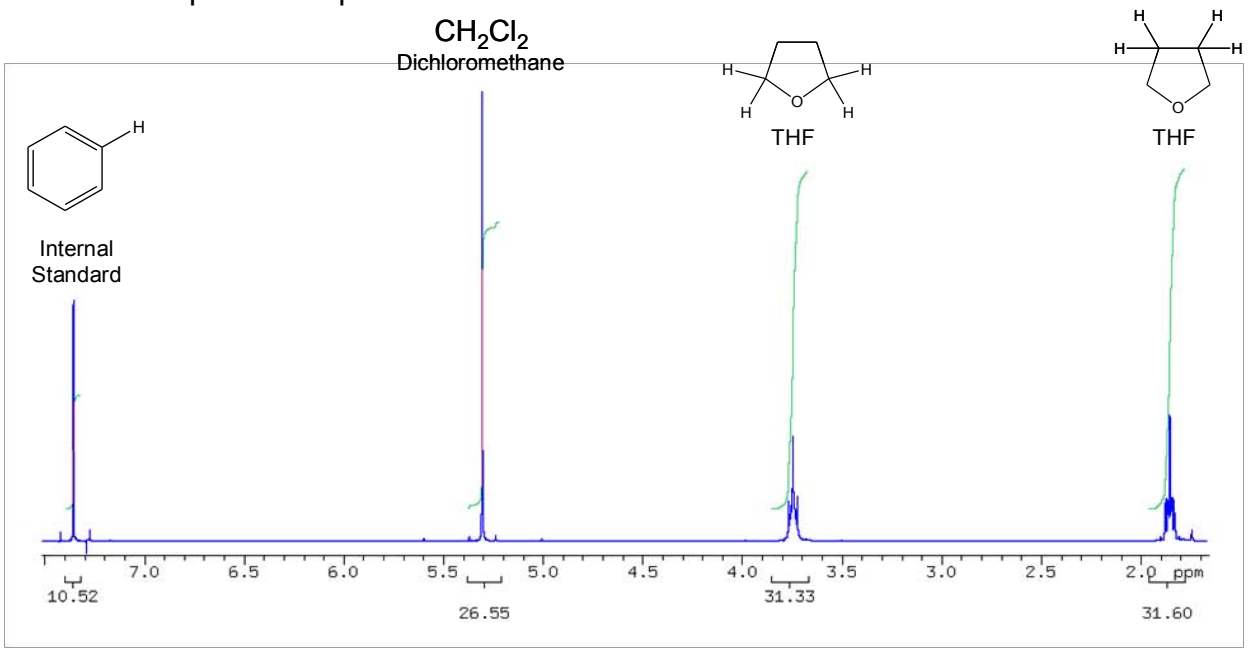


Figure 2. Sample Internal Standard Calibration NMR Spectrum.

In this calibration example, 13.1 mg of THF and 26.5 mg of CH_2Cl_2 were added to a NMR tube containing 0.75 mL of $CDCl_3$ and a benzene / $CDCl_3$ solution sealed in a capillary tube (as described in procedure). The equation used to calculate the capillary calibration constant is shown below. Note that the integrations of THF are added together.

Sample Calculation of Capillary Calibration Constant

$$0.0131\,\text{g THF} \times \frac{1\,\text{mol THF}}{72.1\,\text{g THF}} \times \frac{8\,\text{mol H}}{1\,\text{mol THF}} = 0.00145\,\text{mol H} \Big/ (31.33 + 31.60)\,\text{integ. units} = 2.30 \times 10^{-5}\ \frac{\text{mol H}}{\text{integ unit}}$$

$$0.0265\,\text{g } CH_2Cl_2 \times \frac{1\,\text{mol } CH_2Cl_2}{84.9\,\text{g } CH_2Cl_2} \times \frac{2\,\text{mol H}}{1\,\text{mol } CH_2Cl_2} = 0.000624\,\text{mol H} \Big/ 26.55\,\text{integ. units} = 2.35 \times 10^{-5}\ \frac{\text{mol H}}{\text{integ unit}}$$

The average of these two values is 2.33±0.03 x 10^{-5} mol H / integral unit (1.3% error). The error in these measurements provides a check of student technique. The capillary calibration constant will be in units of mol H (in the capillary) and is calculated as shown below. This calibration constant can vary widely depending on the scaling of the integration values.

$$2.33 \times 10^{-5}\ \text{ave}\ \frac{\text{mol H}}{\text{integ unit}} \times 10.52\,\text{integ. units } C_6H_6\ \text{in capillary} = 2.45 \times 10^{-4}\ \text{mol H in capillary}$$

Alternate Calibration Procedure:

Though the sealed capillary internal standard offers two independent checks on the calibration (referenced to THF and CH_2Cl_2), one could simplify the calibration procedure. This would involve spiking the solvent with a weighed quantity of purified benzene (most likely as a benzene/CD_3OD solution). One could then normalize concentrations based on the benzene integration directly.

Data Analysis:

Processing:

If the data for A (**I+II+III**) and B (**IV**) show significant scattering, check the spectra and integrations in the array. We have found two issues that significantly affect the quantitation. First is the issue of phasing. If the phasing has changed over the course of the data collection, one might need to autophase individual spectra; some could require a more careful manual phasing if autophasing does not produce a flat baseline. Second, some peaks are small and quite close together (e.g. keto-CH_2 and keto-CHD), so the integral ranges need to be set carefully and adjusted if there is any chemical shift difference in the spectra with time.

Fitting Data:

We have had students use an Excel spreadsheet to fit their data 'by-eye' and we have used Origin to do non-linear least squares fits (NLSF). These two approaches have produced essentially the same values for k_1 and k_2 (i.e. good to 2 sig figs).

Using Excel, we have the students insert their experimental data for [A], [B] and [C] as a function of time, then have them calculate the concentrations of each species using Equations 1, 2 and 3. If they place values for k_1 and k_2 in separate cells, they can then refer to them in their equations using absolute addressing. The estimate for k_1 comes from the plot of ln[A] vs. time. The value of k_2 cannot be the same as k_1 or Equations 2-3 will have division by zero errors, but can be essentially the same (e.g.

k_1=1.1 x 10^{-4} s^{-1} and k_2=1.101 x 10^{-4} s^{-1}). If a plot of the experimental and fit data vs. time is inserted on the worksheet, one can alter the values of the rate constants, primarily k_2, and watch the fits change. One can go a step further by calculating an average residual and having the students try to minimize this value.

It may be useful for students to see that within the uncertainty of the data there is little difference whether the data are fit 'by-eye', by manually minimizing the residual, or by NLSF.

A reviewer suggested a slightly more complicated model that might also be considered by students. The rate constants specified are consistent with acetylacetone.

I → **III**	k_1=0.0017		
III ⇆ **IV**	k_2=0.00022	k_{-2}=0.00066	(k_{-2}=3k_2)
IV → **VI**	k_3=0.0017		
VI ⇆ **VII**	k_4=0.00022	k_{-4}=0.00066	(k_{-4}=3k_4)

This assumes:

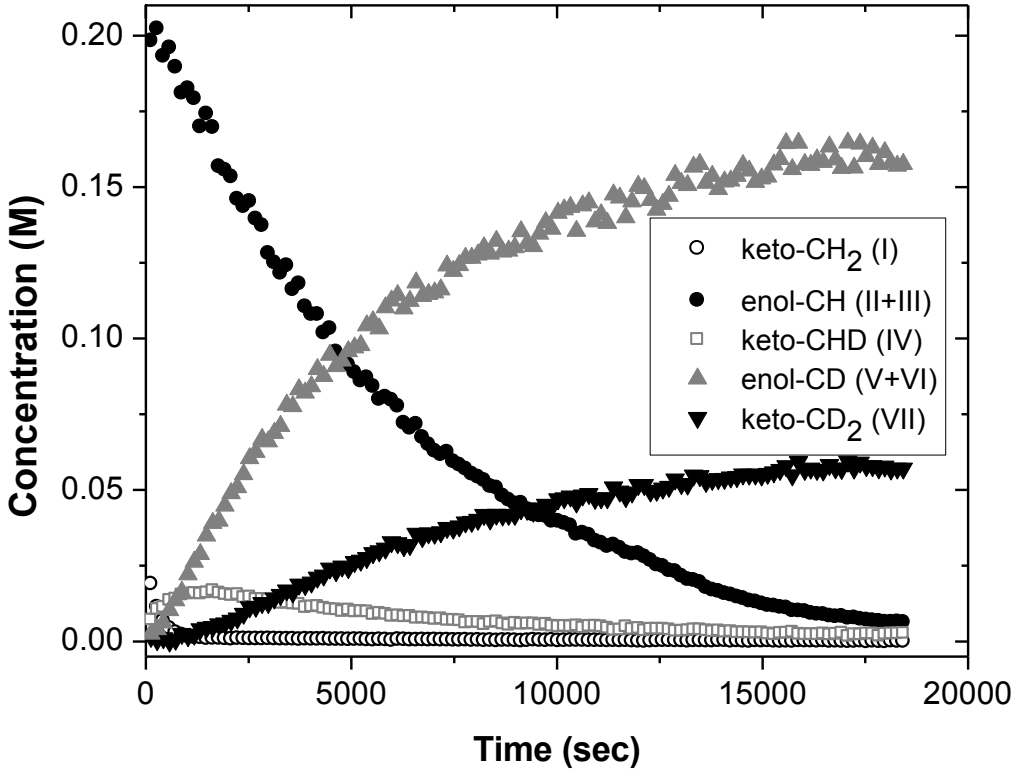
a) **II** → **III** is fast, so **II** and k_{-1} are ignored.
b) **V** → **VI** is fast, so **V** and k_{-3} are ignored.

Figure 3 presents the concentration-time plots for the individual species (**I-VII**) in the acetylacetone and ethyl acetoacetate deuterium exchange reactions.

One can simulate the kinetic data using the Mechanism-Based Kinetics Simulator (http://jchemed.chem.wisc.edu/JCEDLib/WebWare/collection/reviewed/WW007/jceSubscriber/index.htm). This model fits the acetylacetone data quite well. In essence, the reviewer's proposed model simplifies the complex series of equilibria by focusing on the major equilibrium species in the process, namely the enol forms. This results in the conversion of enol **III** to enol **VI**, with the mono-deuterated ketone **IV** as the intermediate. This is very similar to our A → B → C model, as the acetylacetone equilibrium heavily favors the enol form, and therefore, it fits the kinetic data well.

However, when the equilibrium heavily favors the keto form, as it does in ethyl acetoacetate, this model fits species **I** and **III** well, but not species **IV**, **VI** and **VII**. In this case, the enol species (**III** and **VI**, ignoring **II** and **V** as above) now become intermediates in the conversion of **I** → **IV** → **VII**. The mono-deuterated ketone **IV** also behaves as an intermediate in the overall conversion of **I** → **VII**. In fact, the intermediate behavior of species **III**, **IV**, and **VI** is seen in Figure 3b below. Therefore, the overall series of consecutive reactions would be **I** → **III** → **IV** → **VI** → **VII** and the ethyl acetoacetate deuterium exchange would need to be modeled by a more complicated series of consecutive reactions. By combining the keto and enol concentrations together and using the mono-deuterated ketone as an intermediate, our A → B → C model provides a good fit for both systems, regardless of the position of the keto-enol equilibrium.

a)


Concentration (M)
Time (sec)
keto-CH2 (I)
enol-CH (II+III)
keto-CHD (IV)
enol-CD (V+VI)
keto-CD2 (VII)

b)

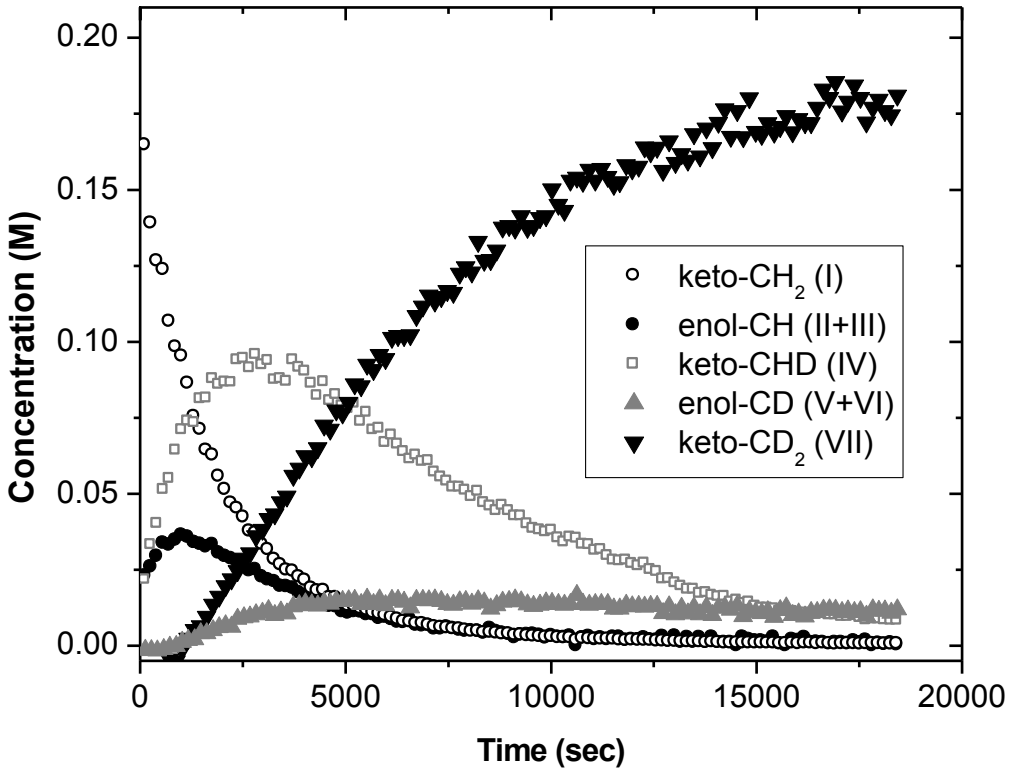
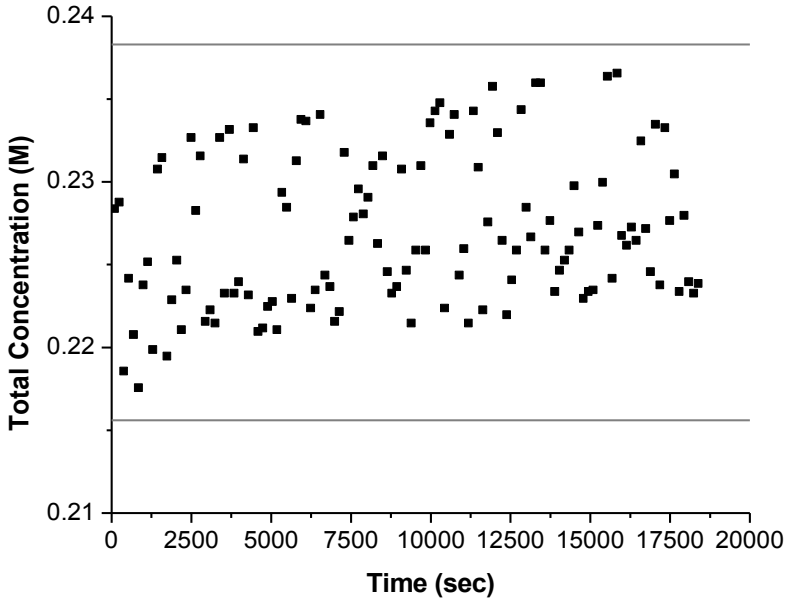

Concentration (M)
Time (sec)
keto-CH2 (I)
enol-CH (II+III)
keto-CHD (IV)
enol-CD (V+VI)
keto-CD2 (VII)

Figure 3. Time Evolution for Observed Species in the Deuterium Exchange Reaction Between a) Acetylacetone and b) Ethyl Acetoacetate and CD_3OD at 25ºC.

Error Analysis:

Figure 4 shows the variation in total (ketone + enol) concentration for acetylacetone and ethyl acetoacetate during the course of the kinetic experiment. The total concentrations were determined from the ketone and enol CH_3 resonances.

(a)



(b)

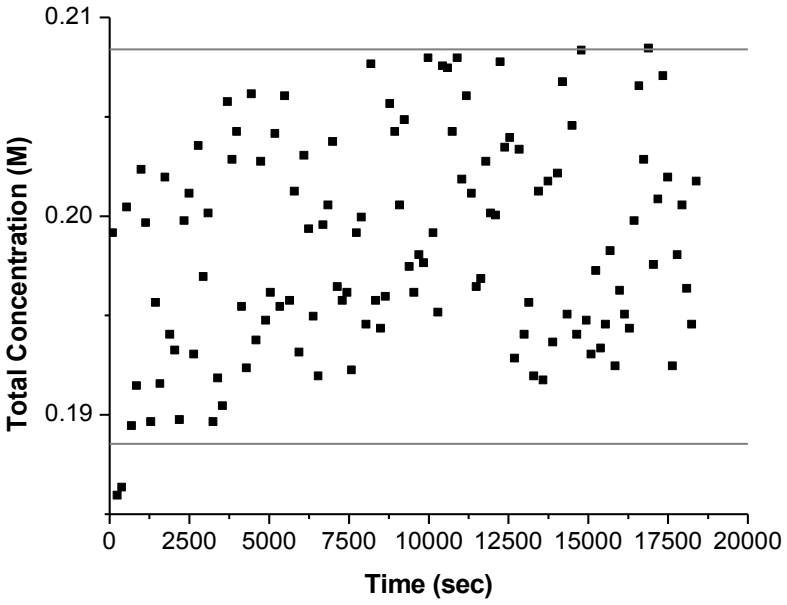


Figure 4. Variation in the Total (Ketone + Enol) Concentrations for (a) Acetylacetone and (b) Ethyl Acetoacetate During the Course of a Kinetic Experiment. The horizontal lines represent ±5% variation around the average concentration.

There is certainly much that can be done with examination of error in this experiment at a variety of levels. Some suggestions are briefly described below:

- Estimation of the uncertainty inherent in the data processing.
    - The same spectrum can be phased and integrated multiple times to estimate the uncertainty inherent in the NMR data processing. We have observed less than 5% variation in peaks of moderate to strong intensity. Very small resonances will obviously yield higher percent variations.
- Sample to sample variability can be examined by replicate measurements, which is time intensive for both collection and analysis of the data.
    - This will include uncertainty in the data processing, as well as variability from the internal standard calibration and measurements of the concentration of each reactant.
    - Our data show that one can expect the values of k_1 and k_2 to be reproducible to two significant digits.
    - Attempts should be made to avoid the introduction of trace acid or base, as this is likely to alter the observed rate constants (i.e. increase them).
- The uncertainty may be measured by examining the standard error introduced by the linear and non-linear regression fits to ln[A] vs. t and [B] vs. t, respectively.

These can be compared to the uncertainties inherent in the concentration measurements.

Additional Kinetics discussion:

If one looks at the overall reaction scheme (see Figure 4 of student handout), one might suggest that species **I**, keto-CH_2, would really be the initial reactant. In the case of ethyl acetoacetate this is a reasonable approximation as $K_{ENOL} << 1$ shows the ketone form is highly favored. Plotting the natural logarithm of the Keto-CH_2 (**I**) concentration vs. time produces a linear relationship over the initial 5.5 hours of the deuterium exchange reaction. Here only small amounts of enol-CH form, but then are drawn off by the deuterium exchange process occurring in large excess of methanol-d_4. As this occurs, the keto-CH_2 (**I**) will react (Le Chatlier) toward reestablishing the equilibrium in a first order way. In the case of acetylacetone, however, $K_{ENOL} > 1$ favors the enol form. Here, keto-CH_2 (**I**) is the dominant species in A throughout the reaction. The analogous ln[keto-CH_2] vs. time plot for acetylacetone displays a non-linear relationship. In this case the relationship consists of a rapid linear decrease (first 27 minutes) followed by a linear decrease with a slope that is two orders of magnitude lower (through 8 hours). Here keto-CH_2 (**I**) is only a minor contributor to A. Plotting the natural logarithm of enol-CH (**II** + **III**) vs. time produces a linear relationship, after an initial 5 minute period, during which the concentration of enol-CH (**II** + **III**) increases.

Question 1 in the student handout can lead to a discussion of kinetic isotope effects for instructors covering this topic, particularly with respect to their effect on equilibrium processes. We and others (see reference 9 in student handout) have not found a significant difference between the $\mathbf{K}^{\mathbf{H}}_{\mathbf{ENOL}}$ and $\mathbf{K}^{\mathbf{D}}_{\mathbf{ENOL}}$ values for acetylacetone and ethyl acetoacetate. Presumably this is a result of the offsetting isotope effects of breaking/making CH(D) and OH(D) bonds in the keto-enol equilibria.

Analysis of data using NUTS:

We have included arrayed NMR spectra for two representative kinetic experiments of acetylacetone and ethyl acetoacetate with CD_3OD at 25°C. They could be used as a "dry" lab if an NMR of sufficient resolution is not available. Each file contains 123 NMR spectra in Varian VnmrJ format. These spectra can be processed, viewed, and integrated using a variety of standalone NMR software products. These files are compatible with either NutsPro – 2D Version[15] or the free demo version of wxNuts.[16]

The two versions of this software have similar commands. To process, view, and integrate the spectra in wxNUTS demo:

1. Start the wxNUTS or NutsPro software.
2. Import the spectra, type **IM <ENTER>** and open the **fid** file in the **sample_acac_cd3od_25oC.fid** or **sample_etacac_cd3od_25oC.fid** folder. The FID of the first arrayed spectrum will appear.
3. Under the **View** menu, select **Processing Parameters**, set **LB** to **0.2**. Click **OK**.
4. Type **EM <ENTER>, FT <ENTER>** and **AP <ENTER>** to apply 0.2 Hz line broadening, Fourier Transform and autophase the spectrum.
5. To access the arrayed spectra, type **VW <ENTER>**. You should see View Slice 1 in the center bottom of the display. Use the **left** and **right arrow keys** to view the individual spectra (slices) in the array.
6. Go to spectrum number 10 in the array. All five observable species should be present in this NMR spectrum. Exit the **VW** routine by pressing **<ENTER>** or selecting **Exit** under the **File** menu.
7. Zoom in on spectrum by typing **ZO <ENTER>**. Hold down the left mouse to zoom in on the region of interest and right mouse click to expand that region. The intensity of the spectrum can be adjusted using the **up** and **down arrow keys.** Exit the **ZO** routine by pressing **<ENTER>** or selecting **Exit** under the **File** menu.
8. Integrate the resonances of interest by typing **ID <ENTER>** and adjust the intensity of the integral markers to be on screen (**[** and **]** keys). The integrals can be broken around each resonance by placing the cursor just to the left of the resonance, clicking on the left mouse button, placing the cursor just to the right of the resonance and clicking on the left mouse button. To integrate the ketone-CH_2 and 1:1:1 ketone-CHD triplet, zoom in on those resonances and repeat the integral process above.
9. Once all the resonances of interest have been integrated for the 10th spectrum, you should not have to change the integral regions throughout the array. You may need to re-phase the spectrum if the integrals become excessively tilted.
10. Enter the **VW** routine and select spectrum (slice) 1. Exit the **VW** routine and enter the ID routine. The integrals of the resonances will appear. The integral

[15] NutsPro - NMR Utility Transform Software – 2D Professional version, Acorn NMR Inc., Livermore, CA, 2005.
[16] wxNuts Demo for Windows – 2nd Generation NMR Utility Transform Software, v. 0.9.4, Acorn NMR, Inc. Livermore, CA, 2007. This can be downloaded at http://www.acornnmr.com/wxNUTS/wxnuts.htm (Accessed Jan. 6, 2010)

list can be copied to the clipboard by either typing **B** or choosing **Integral List to Clipboard** under the **Edit** menu and pasted directly into an Excel spreadsheet.

11. Repeat step 10, integrating and pasting those integral values into the spreadsheet for each spectrum in the array.

Sample data at 25°C:

Included in the online supplemental materials is a complete kinetic array of 123 spectra for acetylacetone and ethyl acetoacetate. If the data are normalized such that the internal standard integrates to 200 units, then the capillary constant will be 2.793×10^{-4} mol H/integral unit. The time and concentration for the first data point is given in Table 2 below.

Time: 145 s[17]	**Enol-CH_3 (M)**	**Ketone-CH_3 (M)**
acetylacetone	0.20	0.028
ethyl acetoacetate	0.021	0.18

Table 2: Concentrations for total ketone and enol forms based on methyl resonances for the first spectrum in the sample data arrays.

[17] This time includes the initial 2 min. delay plus the time required to autolock and acquire the spectrum.