Thermodynamic and Kinetic Characterization of a Hydrolysis Reaction Using the Adjustable Temperature Feature of the Fourier 80

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Innovation with Integrity

Introduction

Benchtop Nuclear Magnetic Resonance (NMR) spectrometers are versatile tools for structure elucidation and verification, reaction monitoring, and quality control. In contrast to their larger, floor standing counterparts they are an order of magnitude less expensive in acquisition, have negligible operating costs, and require only minimal laboratory infrastructure. Although they are naturally less performant than high field instruments in certain aspects, they turn out to be more than adequate for a range of applications and allow NMR methods to be adopted by a much wider community of users.
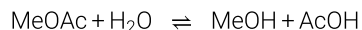
One of the less obvious advantages of floor standing instruments is the possibility to conduct experiments at a wide range of temperatures. A Variable Temperature Unit (VTU) is standard on Bruker spectrometers, and the room temperature iProbes can handle temperatures between – 150 °C and 150 °C by default. The reason why the situation on the benchtop instruments is different is that the permanent magnets used in these instruments are quite sensitive to changes in temperature (on the order of 100 ppm/K). The thermoregulation of the magnet is one of the key challenges when designing such a device, and having a sample cavity that changes in temperature makes this even more difficult. Nonetheless, Bruker is able to offer the Fourier 80 with the Adjustable Temperature (AT) feature which achieves exactly that: the possibility to acquire NMR spectra at a range of different sample temperatures, while keeping the magnet temperature stable at 25 °C.

On the Fourier 80 equipped with AT you can freely adjust the temperature of the sample cavity between 25 °C and 60 °C. After changing the temperature, the magnet will need some time to adapt to the new conditions, depending on the temperature difference. This will range from 4-6 h when changing between the two possible extremes but can be as short as 1 h if one acquires a temperature series and the increments are low (e.g., 5 K between steps). The temperature stability and reproducibility of the sample is on the order of 100 mK. The only additional infrastructure that is needed is a supply of pressurized nitrogen or air. The gas flow box that is regulating the gas flow on the incoming gas is delivered with the system. Furthermore, all Fourier 80 equipped with AT are fully compatible with the automatic sample changer. The additional inlet on the side of the spectrometer also allows the use of a flow cell, which further adds to the capabilities of the Fourier 80 with the AT feature.

There are many different applications imaginable, from polymer science over reaction monitoring to enzyme kinetics. In this application note we showcase the possibilities of this instrument by determining some kinetic and thermodynamic properties of an ester hydrolysis reaction.

Experiment Design

The reaction that we will investigate here is the acid-catalyzed hydrolysis of methyl acetate. In the presence of water methyl acetate splits into methanol and acetic acid, settling into a chemical equilibrium:

Figure 1: Proton NMR spectrum of 1:4 v/v MeOAc / 1M HCl at 25 °C in its hydrolysis equilibrium.

$$MeOAc + H_2O \rightleftharpoons MeOH + AcOH$$

As an acid catalyst we used hydrochloric acid, and we worked with an excess of water to simplify the data analysis step. The concentrations of the reactants can be determined by proton NMR spectroscopy with the Fourier 80. While the acetyl groups of methyl acetate and acetic acid have effectively the same chemical shift (1.43 ppm), the methoxy groups of methyl acetate and methanol can be clearly distinguished in the spectrum at 80 MHz (3.01 ppm and 2.71 ppm, respectively; see Figure 1). These methoxy peaks can therefore be used to monitor the progress of the reaction.

In preliminary experiments, we adjusted the acid concentration so that the reaction would be slow enough to be monitored in real time at all temperatures, but still fast enough to not take excessively long. For a 1:4 ratio by volume of methyl acetate in 1 M HCl the hydrolysis was completed between 30 minutes and 8 hours at the two temperature extremes (60 °C and 25 °C, respectively), which we deemed adequate.

Data Acquisition

For each data point, we set the desired temperature on the instrument and ran the shimming routine during temperature equilibration. We then acquired a spectrum of 99.8% methanol-d_4 to determine the true temperature in the sample tube, using the known relationship between temperature and chemical shift differences between the methyl and hydroxy signals in this reference sample.[1] This is necessary to account for differences between the temperatures at the sensor and inside the sample, but it needs to be done only once for any given instrument.

We then mixed 500 µL of a stock of ~1M HCl with 120 µL of methyl acetate, cross-checking the exact quantities by weighing. The mixture was transferred into an NMR tube fitted with a PTFE valve to avoid any evaporation. The sample was inserted into the Fourier 80 and acquisition was started. As on the Fourier 80 there is no sample-specific tuning/matching, locking, or shimming, the time between mixing and start of acquisition could be achieved in less than a minute. The acquisition consisted of a series of single-scan proton NMR spectra in 30 s intervals.
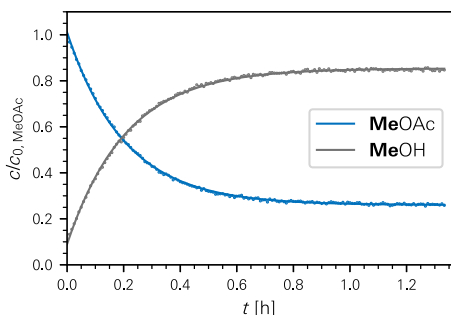

Figure 2: Concentration data for methyl acetate and methanol, scaled to the initial ester concentration, and the exponential fit function. Here the reaction temperature was ~50 °C.
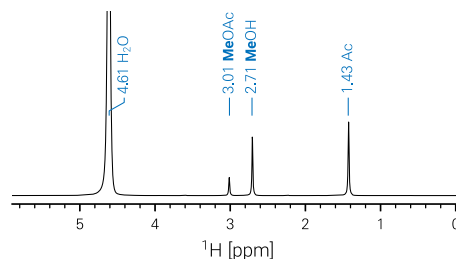
Data Analysis

There are various ways on how to process and analyze the data. Here we chose to use the python interface which communicates with TopSpin via a network interface. This enables us both to customize the analysis to do exactly what we want as well as to automate the entire process, from peak picking and integration via the fitting of the time dependent concentrations to the determination of thermodynamic and kinetic parameters from the temperature dependence. Briefly, the program identified the peak position of the methoxy peaks in each spectrum and integrated a fixed region around these two peaks. We assumed that the rate of change in ester concentration depends in first order on the concentrations of water, acid, and ester:

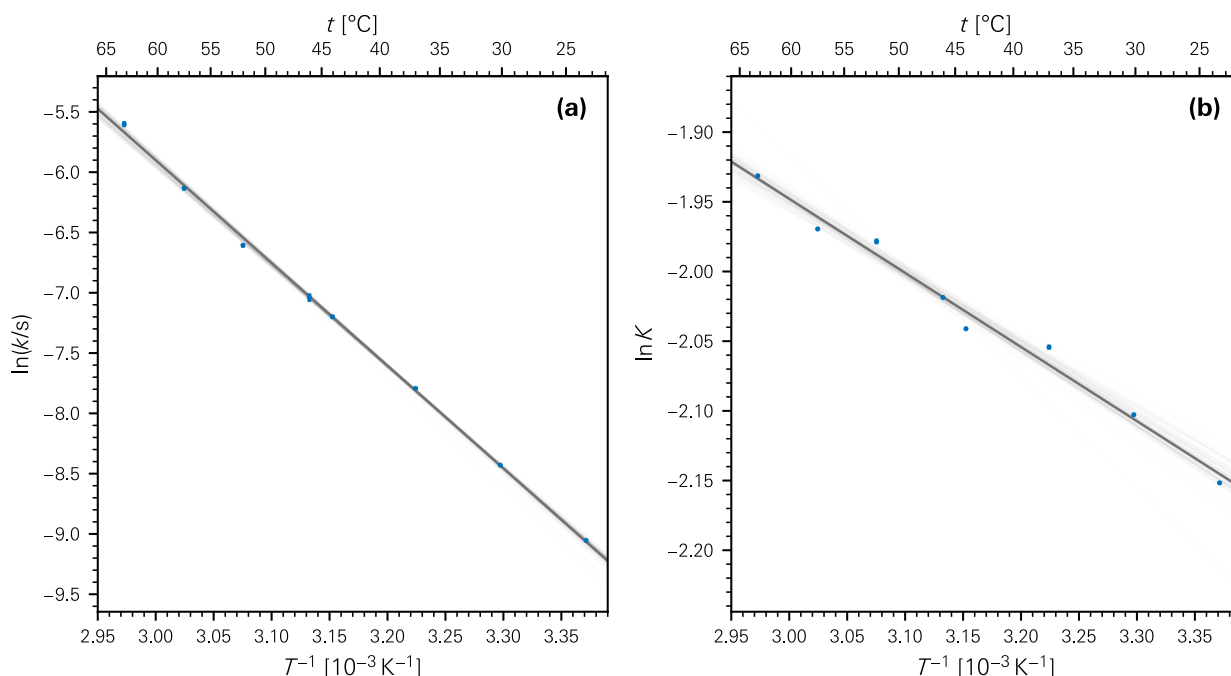$$\frac{d[MeOAc]}{dt} = -k\,[H_2O][H^+][MeOAc]$$

Figure 3: (a) Arrhenius plot of $\ln(k)$ vs $1/T$ and (b) van't Hoff plot of $\ln(K)$ vs $1/T$. The line in dark grey is the least-squares linear fit, while in light grey there is a set of fits from the bootstrap resampling to illustrate the fit uncertainty.

As the acid is not consumed during the reaction and there is an approximately 18-fold excess of water we assumed their concentrations are constant; this leads to a first-order law for the forward reaction (exponential decay). In equilibrium we have about three-fold more products than the ester, which tells us that the backwards reaction is quite a bit slower; we therefore chose to ignore its second-order nature for the moment and fit an exponential decay with offset to the concentration curves:

$$c(t) = (c_0 - c_\infty) \exp(-kt) + c_\infty$$

where $c(t)$ is the concentration as a function of time, c_0 and c_∞ are the concentrations at $t = 0$ and in equilibrium, respectively, and k is the rate constant. As an example, the data including these exponential fits for 50 °C can be seen in Figure 2. From the fits we can also determine the equilibrium constant K:

$$K = \frac{[\text{MeOH}][\text{AcOH}]}{[\text{MeOAc}][\text{H}_2\text{O}]} = \frac{c_{\infty,\text{MeOH}}^2}{18.21 \cdot c_{\infty,\text{MeOAc}} \left(c_{\infty,\text{MeOAc}} + c_{\infty,\text{MeOH}}\right)}$$

In this transformation we made use of some design choices of the experiment. First, the amounts of methanol and acetic acid must be equal since they only originate from the hydrolysis. Secondly, the initial concentration of ester must be the sum of the final concentrations of ester and methanol, and from weighing the reactants, we know that the initial molar ratio of water to ester is 18.21. Lastly, as the number of educts is equal to the number of products, we need not worry about using absolute concentrations in this calculation but can use NMR intensities directly.

We can now use this data obtained at different temperatures to determine some universal characteristics of this reaction. A logarithmic plot of the reaction rate k against the inverse temperature is known as Arrhenius plot and yields the activation energy E_a / R as slope:[2]

$$\ln k = \ln A - \frac{E_a}{RT}$$

Here, R is the gas constant and A is a preexponential factor that we will not discuss any further. This plot can be found in Figure 3a. A simple bootstrap resampling lets us estimate the uncertainty in these results without requiring any assumptions about the nature and distribution of errors in the data.[3] We obtain as result $E_a = 70.8 \pm 1.2$ kJ/mol. Similarly, a van't Hoff plot of $\ln K$ against $1/T$ lets us determine the standard enthalpy and entropy changes ($\Delta_r H^0$ and $\Delta_r S^0$) of the reaction:[2]

$$\ln K = -\frac{\Delta_r H^0}{RT} + \frac{\Delta_r S^0}{R}$$

In the van't Hoff plot, the reaction enthalpy will be the slope and the reaction entropy the vertical intercept, again scaled by the gas constant R. The plot can be found in Figure 3b. The result of this analysis is $\Delta_r H^0 = 4.42 \pm 0.21$ kJ/mol and $\Delta_r S^0 = -2.93 \pm 0.67$ J/mol/K.

An Example of More Sophisticated Data Analysis

Given that all data handling was done with python using the extensive scipy ecosystem,[4] it is rather straightforward to modify the analysis to incorporate the true second-order nature of both the forward as well as the backward reaction. With c_0 being the starting concentration of the ester and $f = 18.21$ being the excess of water relative to the water, the rate law for the concentrations of the ester c_e is as follows:

$$\frac{dc_e}{dt} = -k_1 [\text{MeOAc}][\text{H}_2\text{O}] + k_2 [\text{MeOH}][\text{AcOH}] = -k_1 c_e (f c_0 - c_0 + c_e) + k_2 (c_0 - c_e)^2$$

$$\frac{dc_e}{dt} = (k_2 - k_1) c_e^2 + ((1 - f) k_1 - 2k_2) c_0 c_e + k_2 c_0^2$$

Here, k_1 and k_2 are the rates of the forward (hydrolysis) and backward (esterification) reactions, respectively. Similarly, the rate law of the methanol concentration c_m can be derived:

$$\frac{dc_m}{dt} = (k_1 - k_2) c_m^2 - (1 + f) k_1 c_0 c_m - f k_1 c_0^2$$

These are quadratic first-order differential equations with a known solution:

$$\frac{dc}{dt} = \alpha c^2 + \beta c + \gamma$$

$$c(t) = -\frac{1}{2\alpha}\left(k_{\text{eff}} \tanh\left(\frac{1}{2}k_{\text{eff}}(t - t_0)\right) + \beta\right), \quad \text{with } k_{\text{eff}} = \sqrt{\beta^2 - 4\alpha\gamma}$$

With these results in place, we can use a non-linear solver from scipy's optimization module to do a simultaneous fit of both concentration curves, yielding us the global optimum of the parameters k_1, k_2, c_0 and t_0. The equilibrium constant is now simply the ratio of the two rates $K = k_1 / k_2$, and we can again make Arrhenius and van't Hoff plots (Figure 4). We get $E_{a1} = 73.0 \pm 1.4$ kJ/mol, $E_{a2} = 68.3 \pm 1.4$ kJ/mol, $\Delta_r H^0 = 4.64 \pm 0.16$ kJ/mol and $\Delta_r S^0 = -1.91 \pm 0.49$ J/mol/K. This allows us to properly characterize the energy landscape with the height of the barrier in both directions instead of a rather poorly defined mix of the two. The reaction enthalpy is obviously the difference between the two and is therefore not independent, but it has a much smaller absolute uncertainty due to error correlation in k_1 and k_2. Overall, we also can see this analysis yields slightly smaller uncertainties for the reaction enthalpy and entropy.
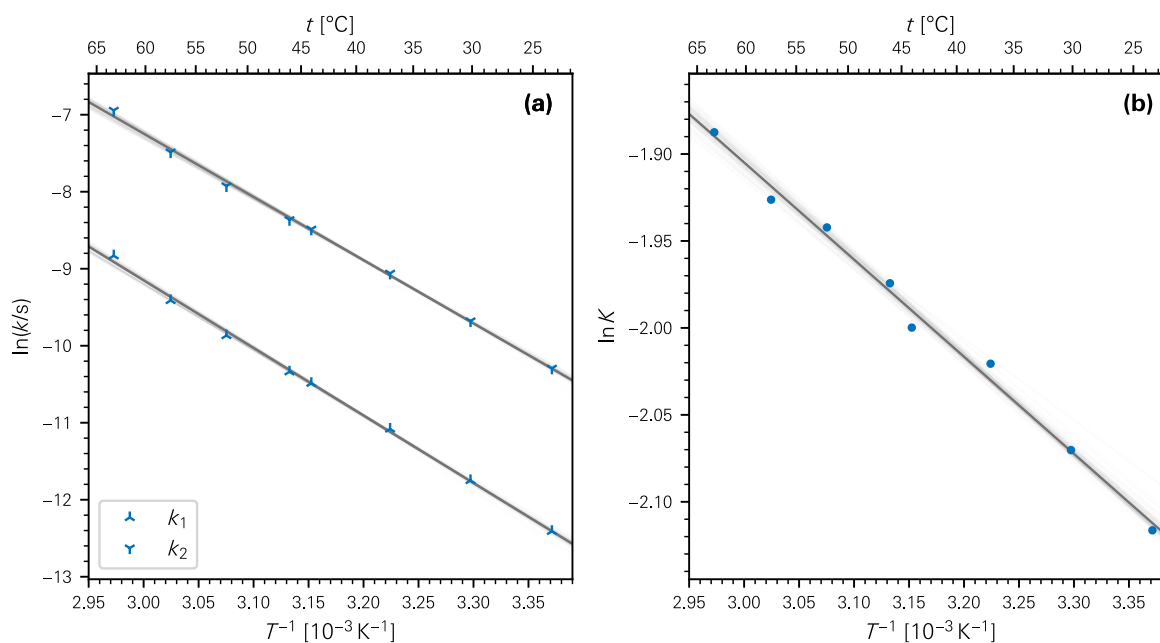
Figure 4: (a) Arrhenius plots of k_1 and k_2 and (b) van't Hoff plot. This data comes from the fits taking into account the second-order nature of the reaction.

Concluding Remarks

In this showcase example, we demonstrate one possible use case of the Fourier 80 equipped with Adjustable Temperature (AT). By following a reaction at different temperatures, we could determine some thermodynamic and kinetic parameters of this reaction. We have confirmed in these experiments that the system offers good temperature stability (once equilibrated, the sample temperature does not fluctuate) as well as reproducibility (when changing back and forth between different temperatures, the equilibrium temperatures are always the same). Despite the smaller temperature range when compared to high field instruments with VT units, the Fourier 80 with AT is perfectly capable of accurately measuring thermodynamic and kinetic parameters via a temperature series, and it contributes considerably to expand the usability of benchtop NMR spectrometers.

References:

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4. Virtanen, P.; et al. SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat. Methods* **2020** *17*, 261–272. DOI: 10.1038/s41592-019-0686-2