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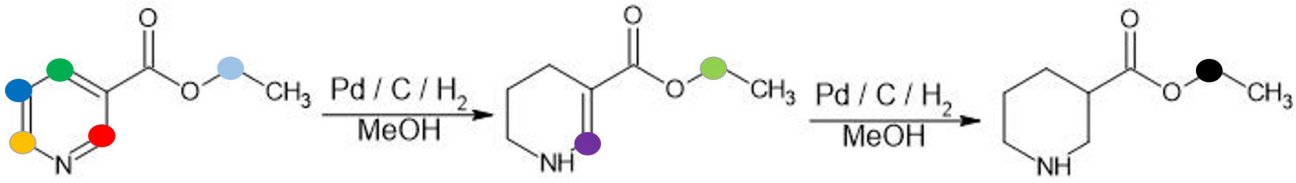
Optimizing a two-step hydrogenation reaction in a flow reactor using a Spinsolve Benchtop NMR

Spinsolve 80 Ultra monitoring the hydrogenation reaction performed with an H-Cube® Pro from ThalesNano.

Introduction

In a recent blog post we demonstrated that coupling a Spinsolve benchtop NMR spectrometer to a continuous flow reactor can dramatically accelerate the optimization of the reaction parameters. Benchtop NMR spectroscopy provides online access to quantify the reaction conversion in real time as the reaction parameters are adjusted. The example chosen in the previous work to test the setup and the performance of benchtop NMR was the hydrogenation reaction of the double bond in cinnamyl alcohol. In that case, the quantification of starting material and product was straightforward. The simplicity of the NMR spectra of those compounds was useful to evaluate the reproducibility and robustness of the NMR method. In this application note, we explore the power of this setup to optimize the more complex two-step hydrogenation reaction of the hetero-aromatic ring of ethyl nicotinate (see Scheme 1) as a function of temperature, pressure, equivalents of H2, and flow rate.



Scheme 1: Hydrogenation of Ethyl nicotinate in two steps using methanol as a solvent at a concentration of 50 mM.

Experimental Setup

For this experiment, the H-Cube® Pro from ThalesNano was equipped with a 10% Pd/C cartridge and an HPLC pump to control the flow of the starting material solution. As starting material, we prepared a 50 mM solution of ethyl nicotinate in methanol (2.26 g/15 mmol in 300 mL). A Zaiput SEP10 gas-liquid phase separator was employed at the output of the reactor to eliminate residual hydrogen gas in the solution. To monitor the reactor's output online, a Spinsolve 80 Ultra Multi-X benchtop NMR spectrometer fitted with a reaction monitoring kit 2 (RMK2) was used. The RMK2 includes a flow cell that enables for continuous pumping of the reaction mixture through the Spinsolve.
For ^{1}H-NMR measurements, the 1D ^{1}H WET sequence with 8 scans and a repetition time of 10 seconds was employed. The WET sequence was configured to suppress the two methanol signals at 3.3 ppm and 4.9 ppm. The NMR signals were collected in the presence of carbon decoupling to eliminate carbon satellites of the protonated solvent from the spectra. The reaction mixture was monitored every 2 minutes.

Results

A key to extracting quantitative information from the NMR spectra of samples dissolved in regular protonated solvents relies on the effectiveness of the solvent suppression method. The WET sequence implemented on the highly homogeneous magnetic field of the Spinsolve ULTRA efficiently attenuates the large signals of the protonated solvents, which would otherwise overlap with the smaller signals of the starting material and products. To demonstrate the power of this method, Figure 1 compares the spectra of the starting material acquired with a standard 1D and the WET suppression with carbon decoupling sequences. The top spectra in the stack plot are presented in full scale, while the spectra at the bottom are zoomed in for a more detailed view.

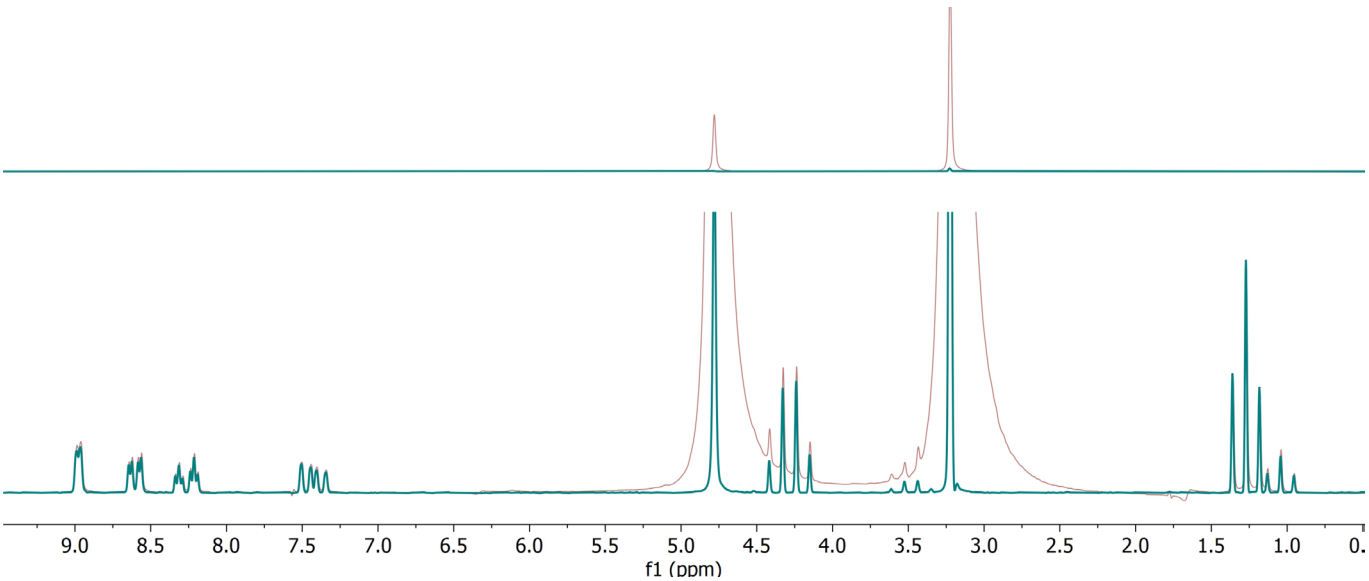


Figure 1: Comparison of the spectra of the starting material acquired with a standard (red) and a WET suppression sequence (blue). Both spectra were acquired in the presence of carbon decoupling. These measurements were performed in continuous flow mode with a flow rate set to 1mL/min.

To track the hydrogenation process of ethyl nicotinate to its intermediate and, subsequently, the hydrogenation to its final product using NMR spectroscopy, there are two distinct regions in the spectrum where the signals change. Initially, our attention was directed to the aromatic section of the spectra. This region encompasses the pyridine residue and exhibits four signals for the starting material, corresponding to the four protons in the hetero-aromatic ring. These four signals are marked with different colors in the spectra shown in Figure 2.

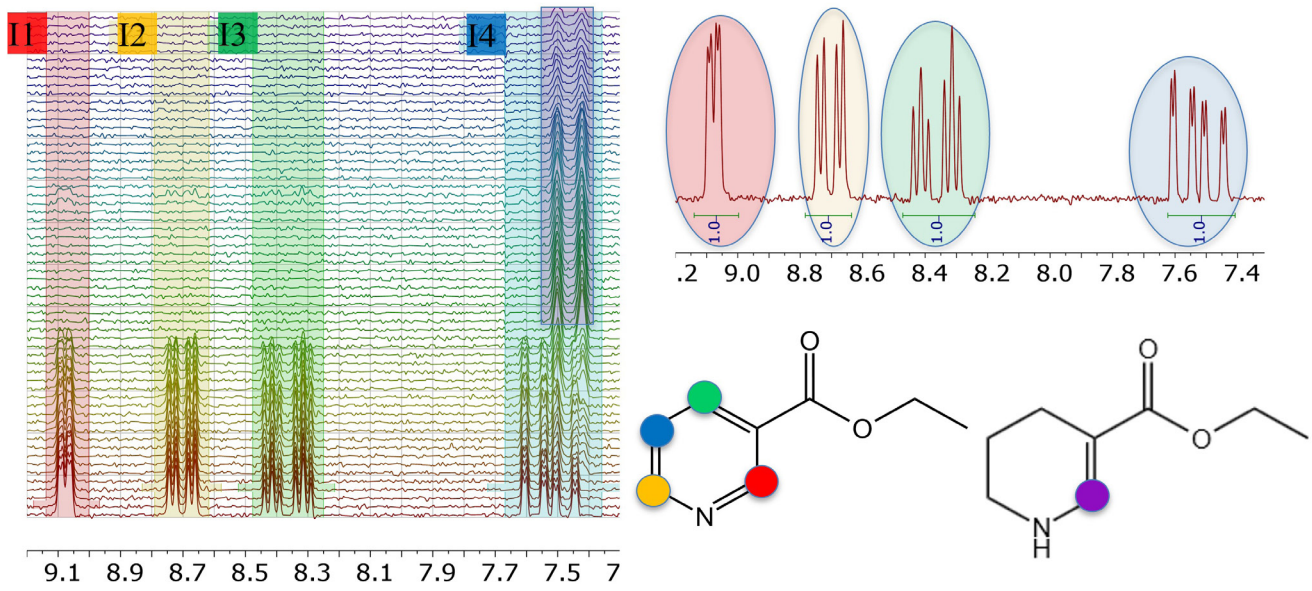


Figure 2: Aromatic signals of the starting material and Intermediate.

The monitoring experiment started with pumping the starting material exclusively through the reactor without hydrogen production. Hence, only signals from the starting material are observable in the initial spectra. The integral regions 1 (red), 2 (yellow), 3 (green), and 4 (blue), correspond to the different hydrogen nuclei in the pyridine ring. When hydrogen is produced at low concentrations (mild reaction conditions) the reaction is activated, but only the intermediate is formed. In this scenario, only the first two of the three double bonds in the aromatic system of the starting material are reduced, leaving one double bond in the intermediate. In the NMR spectra, integrals 1-3 vanish in the aromatic area, as the newly formed CH2 groups shift to the higher field (between 2-3 ppm). The higher shifted CH group of the remaining double bond, marked in violet, appears at 7.5 ppm, overlapping with the signal of the blue CH group in the structure of the starting material (Integral region 4). Despite this overlap, the concentration of the intermediate can be calculated by subtracting from I4 the contribution of the starting material obtained from I1. The signal of the proton of the starting material contributing to the blue region (I4) is equivalent in magnitude to one of the other three protons in the positions marked red, yellow, and green. By using the integral 1 (red), the concentration of the intermediate can be calculated as

$$c_{IM}(t) = \frac{I_4(t) - I_1(t)}{I_1(0)} c_{SM}(0) \tag{1}$$

To convert the integral to concentration we calibrate the value of Integral 1 obtained for the starting material before the reaction, which was prepared with a known concentration. Eq. 1 delivers the concentration of the Intermediate at any time during the reaction. As shown below, a second alternative to calculate the concentration of the intermediate is to use the respective signal in the alkyl area. To determine the concentration of the remaining starting material we use Integral 1. To convert to concentration, we simply normalize by the initial integral corresponding to the known concentration in the starting solution, as shown in the following equation.

$$c_{SM}(t) = \frac{I_1(t)}{I_1(0)} c_{SM}(0) \tag{2}$$

As the product is fully hydrogenated, it has no signal in the aromatic region of the spectra. To monitor the concentration of the final product we need to focus on a second region in the spectrum. All three components share the same ethyl residue, which consistently manifests as one quartet and one triplet. However, these quartet and triplet patterns experience slight shifts depending on the residual structure. Figure 3 shows a zoom of the spectra between 3.8 and 4.6 ppm where the quartet of all three components can be observed.

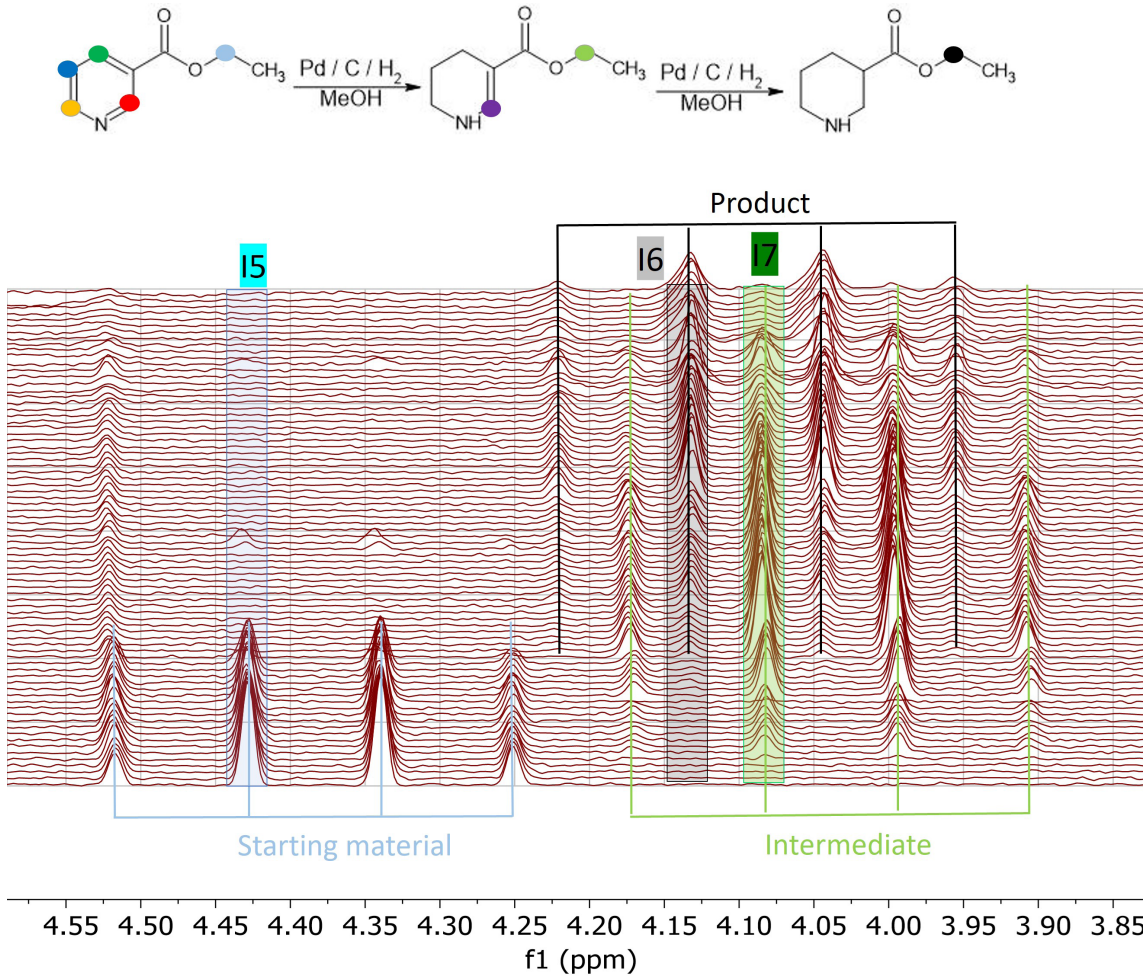


Figure 3: Ethyl area of starting material, Intermediate, and Product.

The quartet of the starting material, marked with light blue lines, is well separated from the quartets of the intermediate and product. Still, one of the signals slightly overlaps with an impurity on the left. Therefore, we only used one of the four peaks of the quartet for integration (marked in red). The quartets associated with the product and the intermediate, displayed in black and green, respectively, are shifted from each other enough to resolve the individual amplitudes of each quartet. These signals can be resolved so well, thanks to the high homogeneity of the ULTRA magnet. This version of the Spinsolve magnet defines very narrow linewidths, enabling the close quartets to be separated. The fact that an 80 MHz spectrometer was used, instead of a 60 MHz, also helped separate these two quartets. The well-separated peak marked in black, was employed to calculate the concentration of the product at any given time using the following equation.

$$c_{Pr}(t) = \frac{I_6(t)}{I_5(0)} c_{SM}(0) \tag{3}$$

Figure 4 shows four spectra extracted from the main stack plot of the reaction monitoring experiment. The spectra were zoomed in to show the three regions of interest. The bottom spectrum corresponds to the starting material. The ratio of integrals for all aromatic signals is approximately 1:1. The second spectrum corresponds to an experimental condition where 50% conversion towards the intermediate was achieved, which can be observed by integrating the corresponding quartets of the initial and intermediate products. The third spectrum shows the starting material's complete depletion (no product signal can be observed in the aromatic region). The intermediate signal is approximately 1, indicating 100% conversion towards the intermediate and no presence of the final product. Finally, the fourth spectrum shows complete depletion of the starting material and conversions close to 60% of the final product and 40% of the intermediate. The stack plot effectively showcases how clean the signals of the different species can be resolved to monitor the two-step reaction.

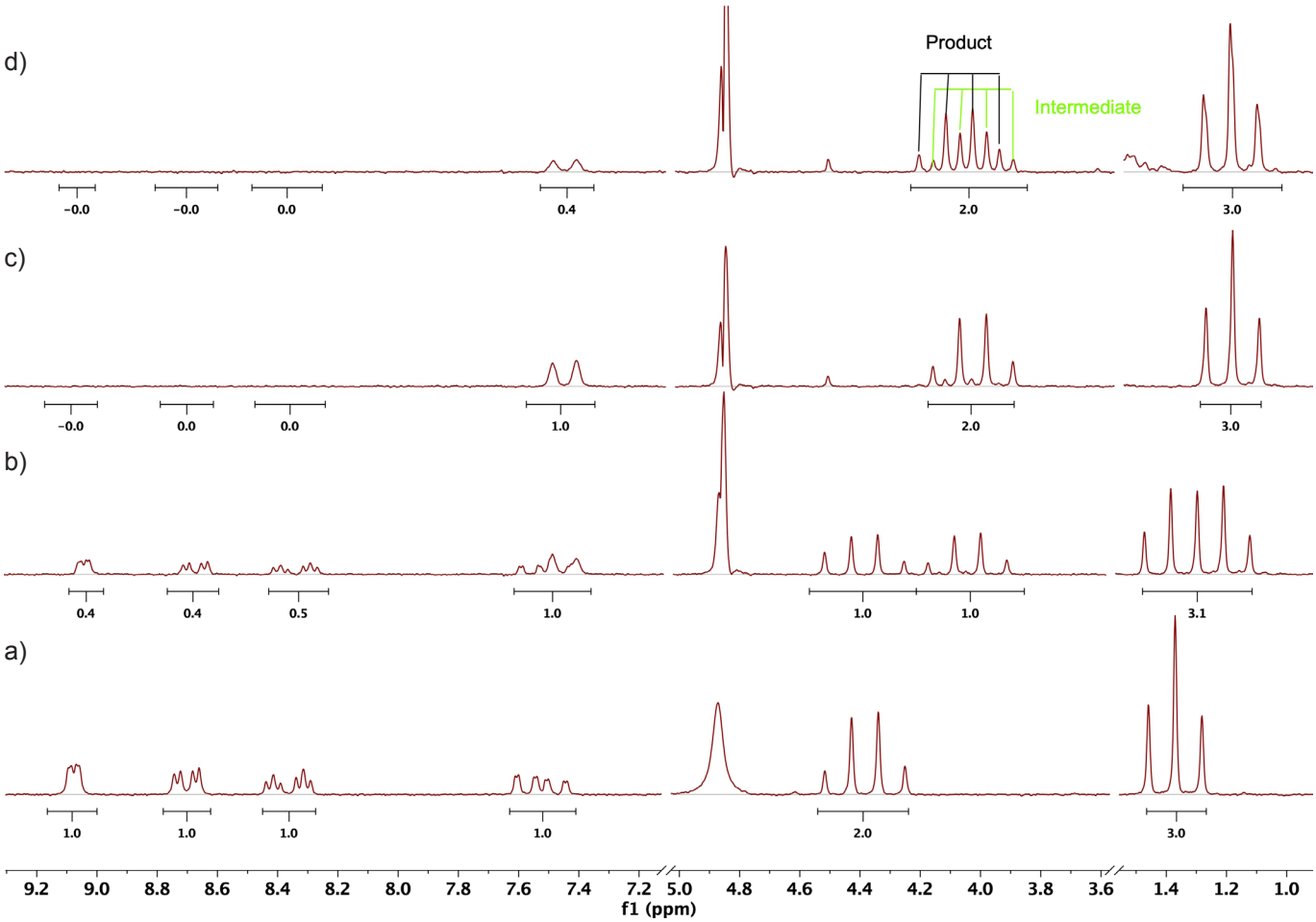


Figure 4: Stack plot of spectra extracted from the reaction monitoring experiment under different experimental conditions. a) Only starting material signals. b) Starting material and intermediate at about 50% each. c) Intermediate at about 100%. d) Intermediate and product at about 40% / 60%, respectively.

Figure 5 shows the concentrations of starting material, intermediate, and final product for different temperatures, pressures, and hydrogen concentrations in the reactor. To determine the concentration of the starting material and intermediate we used the signals in the aromatic region (see eq. 1 and 2), while for the concentration of the final product we used the respective CH_2 signals (black region in Fig. 3).

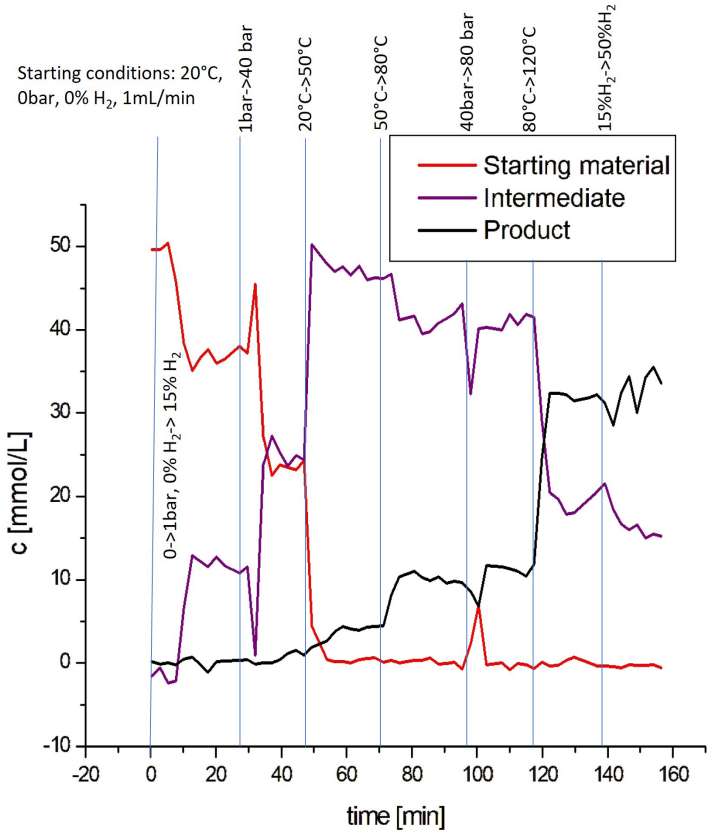


Figure 5: Concentration of starting material (red), intermediate (violet), and product (black) measured for different values of pressure, temperature and hydrogen concentration in the reactor.

In summary, it can be concluded that a more complex two-step hydrogenation reaction can be easily monitored by coupling the continuous flow reactor to a Spinsolve benchtop NMR spectrometer. The conversion towards the intermediate and final product is automatically calculated and displayed in the reaction monitoring module of the Spinsolve software. It is noteworthy to mention that all the measurements presented in this app note have been performed with the WET solvent suppression sequence with the sample flowing in continuous flow mode. The high performance of the suppression confirms the high stability of the Spinsolve benchtop NMR throughout the entire duration of the reaction monitoring experiment.