



## Chemical Kinetics by $^{13}\text{C}$ DNP NMR

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■ The huge enhancement of the signal to noise ratio (SNR) afforded by DNP NMR allows direct detection of several low abundance nuclei (for example,  $^{13}\text{C}$  or  $^{15}\text{N}$ ) in a single scan. By using small flip angles the enhanced magnetisation can be monitored over time to follow kinetic processes. An example is shown here for a chemical reaction.

### Introduction

One of the main limitations of NMR spectroscopy, in particular when applied to heteronuclei, is its relatively low sensitivity. This is caused by the low natural abundance of the respective NMR active isotopes (e.g. 1% for  $^{13}\text{C}$ , 0.037% for  $^{15}\text{N}$ , etc) and by the low polarisation of the sample. The extent of polarisation of a given nuclei is a ratio of the magnetic moment to the thermal energy and is linked to the relative populations of spin states, which is typically in the region of 5-20ppm, and reflects the very small amount of sample that actually contributes to the NMR signal.

One way of increasing the sensitivity of NMR is to create an enhanced, non-Boltzmann spin state population, resulting in enhanced NMR signals. The approaches used to achieve such enhanced NMR signals are generally referred to as "hyperpolarisation techniques", and

perhaps the most generally applicable is Dynamic Nuclear Polarisation (DNP). DNP has been shown to yield signals for  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclei in solution-state NMR that are enhanced by over 10,000 times in comparison with conventional NMR spectra<sup>1</sup>.

The technique involves cooling a sample to <4K in a strong magnetic field ( $B_0 = 3.35\text{T}$ ) in the presence of a trityl radical<sup>2</sup>. Under such conditions, the unpaired electrons on the trityl radical become strongly polarised, and this polarisation can be transferred to nearby atomic nuclei using microwave irradiation ( $\nu \cong 94\text{GHz}$ ). Once the polarisation has built up to a sufficient level, the sample is dissolved by the injection of an aliquot (typically 3–5mL) of hot solvent and rapidly (<1s) transferred to a conventional NMR spectrometer for measurement.

In this article, we show that the gain polarisation afforded by  $^{13}\text{C}$  DNP NMR can be used not just for high sensitivity but can be read out in a time wise fashion to study kinetics in real time.

### Experimental considerations

For DNP measurements, solid samples were dissolved in a mixture of methanol:DMSO (1:1, v/v). A trityl radical (15mM) was added to act as the source of free electrons. The sample was then immersed in super cooled liquid helium ( $T = 1.4\text{K}$ ) in a 3.35T magnetic field and irradiated with microwaves ( $\nu \cong 94\text{GHz}$ ) (see below). After polarisation, the samples were dissolved in 4mL of hot methanol (~140°C) and rapidly (<1s) transferred to a conventional 400MHz NMR spectrometer for measurement for recording a  $^{13}\text{C}$  NMR spectrum.

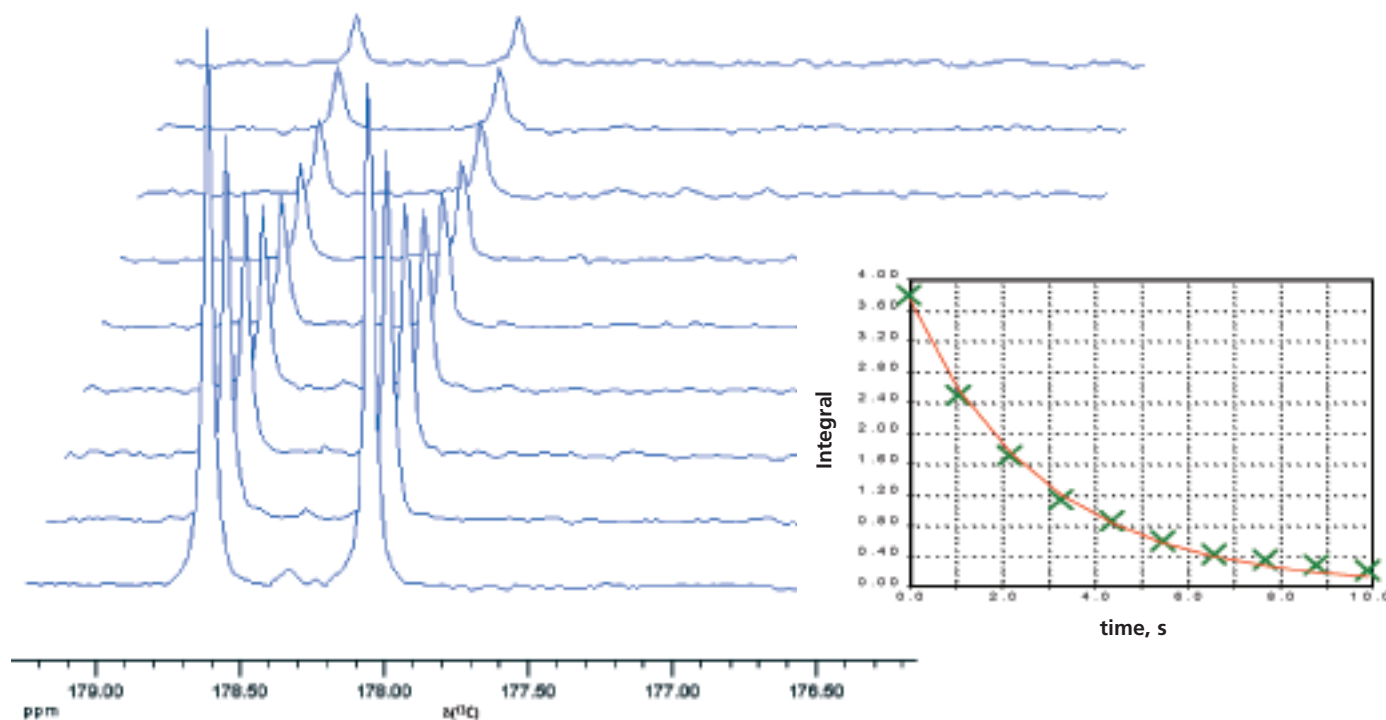
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## Results and discussion

### Real time $^{13}\text{C}$ spectra

The greatly increased SNR makes it feasible to acquire  $^{13}\text{C}$  DNP NMR spectra by using a single low tip-angle pulse. In this way, if the  $^{13}\text{C}$   $T_1$  is sufficiently long, a number of consecutive spectra can be acquired from a single hyperpolarised sample in quick succession. An example of real time  $^{13}\text{C}$  acquisition is shown for U- $^{13}\text{C}$ -sodium acetate, see figure 1.

The labelled ethyl pyruvate carboxyl carbon is clearly visible at  $\delta 160.5$ . A second resonance is also visible at  $\delta 171.8$ , and can be attributed to the protonation of the alpha-keto group by the solvent, which can be followed by enol formation<sup>3</sup>. It is interesting to note that the reaction is fast, but not instantaneous: it takes approximately 3 seconds for the equilibrium between the protonated and non-protonated forms to be established.

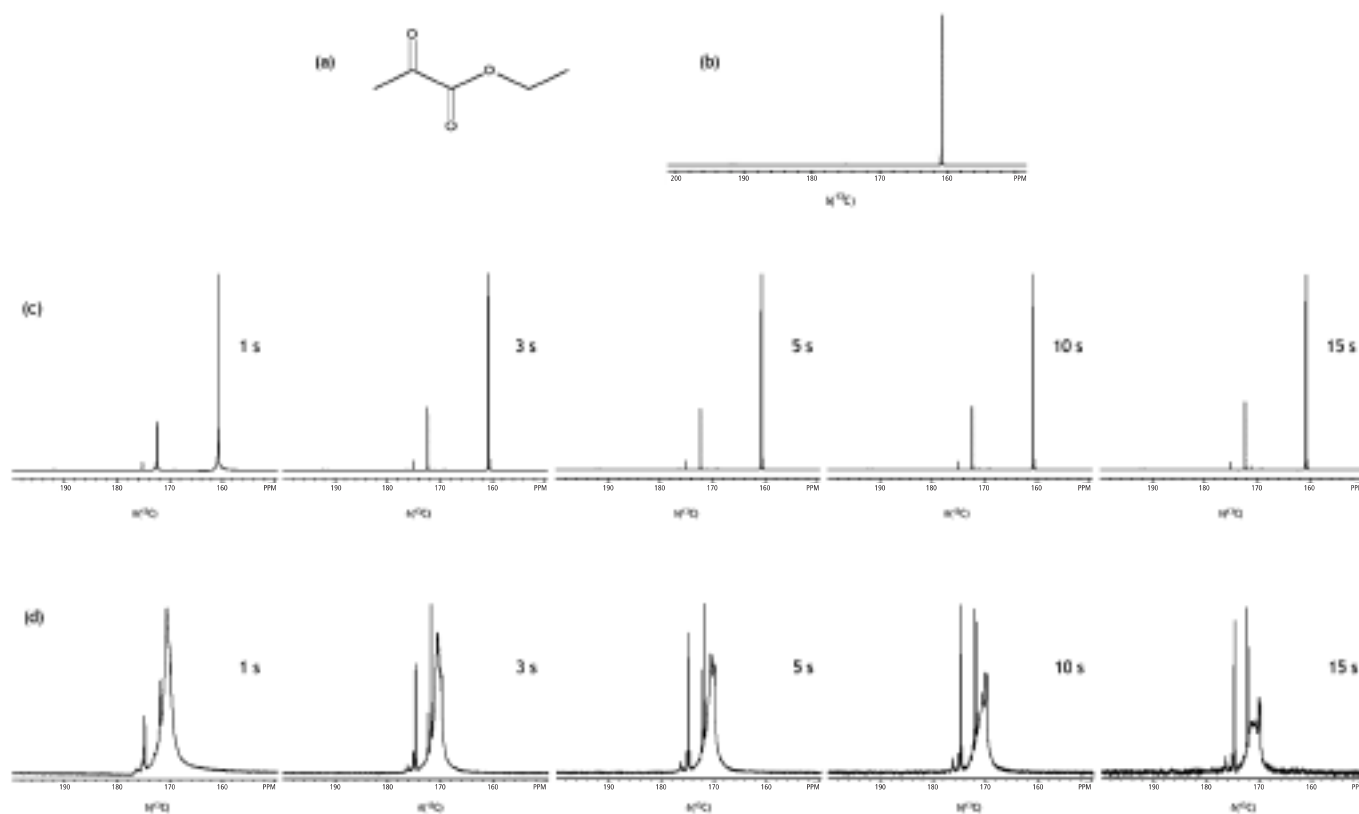


**Figure 1:**  $^{13}\text{C}$  single scan acquisition (45 degree pulse) showing the carbonyl region of U- $^{13}\text{C}$ -sodium acetate measured every 1.1 seconds. Inset shows the decaying peak integral due to relaxation and rf pulse.

### Study of Chemical Kinetics

The acquisition of real time NMR spectra permits the monitoring of kinetic processes. As an example the hydrolysis of 1- $^{13}\text{C}$ -ethyl pyruvate is shown (Figure 2a and 2b). In Figure 2c, the sample was polarised for 100 minutes followed by dissolution using methanol.

Prompted by this observation, we attempted to study a slower reaction *in-situ* by DNP: the hydrolysis of ethyl pyruvate. The sample was polarised for 100 minutes and dissolved in methanol, followed by transfer into a 5mm NMR tube containing 50 $\mu\text{l}$  of 75mM aqueous solution of sodium hydroxide. In this way, the first pulse could be applied as soon as the transfer of pyruvate was complete, and the reaction monitored *in-situ* almost immediately after it was initiated. Further spectra were then acquired at a rate of one per second. Representative examples are shown in Figure 2d.



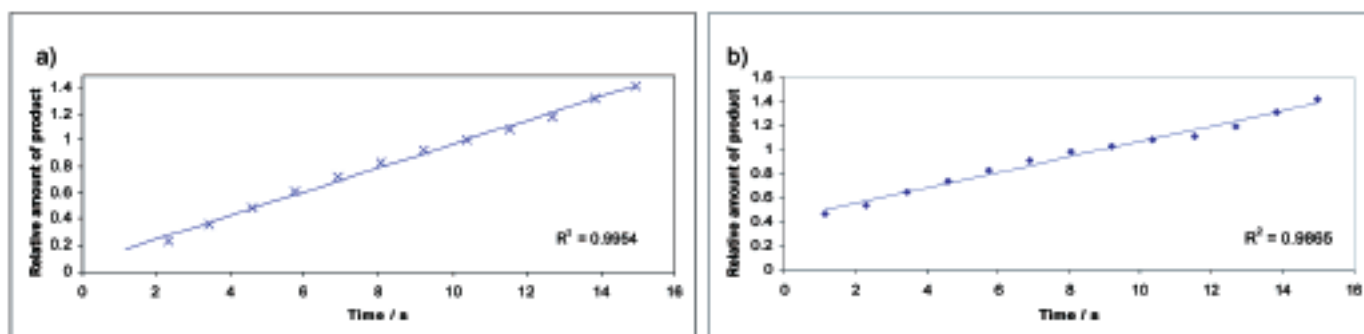
**Figure 2:** (a) Structure of 1-<sup>13</sup>C-ethyl pyruvate, (b) Carbonyl region of a one scan conventional <sup>13</sup>C NMR spectrum of 750μl of neat material, (c) Carbonyl regions of consecutive DNP NMR spectra of 50μl of ethyl pyruvate, polarised for 100 minutes and dissolved with methanol, (d) Carbonyl regions of consecutive DNP NMR spectra of 50μl of ethyl pyruvate, polarised for 100 minutes and dissolved with methanol into a 75mM aqueous solution of NaOH.

Although the early spectra in this reaction (Figure 2d) are affected by the presence of a very broad resonance, a number of useful deductions can be made about the reaction. As expected, the enol formation is catalysed by the presence of NaOH<sup>3</sup>, as evidenced by the complete absence of the resonance at δ160.5, whilst δ171.8 is still present. The enolate species then undergoes two main reactions, as evidenced by the build-up of two new

resonances at δ172.3 and δ174.7. These are tentatively assigned to the hydrolysis of the ester group and to transesterification by the methanol dissolution solvent<sup>4</sup>.

It is possible to extract kinetic information for both reactions. The  $T_1$  relaxation times of the three carbon atoms were measured to be identical within experimental error ( $37 \pm 3$  s). The only correction that needs to be made is therefore for the effect of the NMR excitation pulses, which can be done simply by plotting the ratio of each product to the reactant as a function of time. The resulting charts are shown in Figure 3.

The data in Figure 3 demonstrate that both reactions are of the first order, and rate constants of  $(91 \pm 4) \times 10^{-2} \text{s}^{-1}$  and  $(64 \pm 4) \times 10^{-2} \text{s}^{-1}$  can be extracted by linear regression analysis for the resonances at δ172.3 and δ174.7, respectively.



**Figure 3:** Plots of reaction progress for the hydrolysis of ethyl pyruvate as detected by DNP NMR: (a) product resonating at  $\delta$ 172.3; (b) product resonating at  $\delta$ 174.7.

The data presented above clearly demonstrate the feasibility of using DNP NMR to monitor the progress of chemical reactions *in-situ* at high sensitivity. However, some unknowns still remain, including the full characterisation of all reaction products and the identification of the broad resonance, which may be a reaction intermediate.

## Conclusions

DNP can dramatically increase the signal-to-noise ratio of NMR spectroscopy. The signal is sufficient such that the polarisation can be read out in a time wise fashion. This makes it possible to use <sup>13</sup>C NMR in the currently unusual situation of monitoring kinetics in real time at high sensitivity. The time period over which kinetics can be probed is dependent upon the T<sub>1</sub> relaxation time of the nuclei being observed.

## Possible uses could included:

- Catalysis
- Enzyme turnover
- Tautomerism

## References

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