

Introduction

To apply the benefits of Dynamic Nuclear Polarisation (DNP) to NMR spectroscopy, we expose samples to a 3.35 T magnetic field at very low temperature (< 4 K) in the presence of a trityl radical and a glassing agent.¹ Under such conditions, the free electrons on the radical become almost fully polarised. This polarisation is transferred to atomic nuclei by microwave irradiation at an appropriate frequency (ca. 94 GHz from a commercially available source). The hyperpolarised sample is then rapidly dissolved in a hot solvent and transported into the magnet of a high field NMR spectrometer, yielding greatly enhanced resonances in solution-state NMR spectra.¹ In theory, such samples are limited to a single scan observation (using a 90° pulse) before the polarisation needs to be repeated.

The first dissolution solvent used for this purpose was water.^{1,2} In this poster we demonstrate that methanol can be an interesting alternative for a number of applications. As one of the simplest organic solvents, methanol can dissolve a number of hydrophilic and hydrophobic compounds. Its low viscosity and surface tension, as well as a very low freezing point (-98°C) make methanol suitable for DNP dissolutions and very reliable for filling both 10mm and 5mm NMR tubes. From a spectroscopic perspective, methanol exhibits only a single ¹³C resonance at ca. δ 50.0, minimising the risk of spectral overlap with the sample.

We further show that DNP samples can exhibit polarisation transfer in the low-field environment outside the NMR magnets, and that multiple NMR spectra can be acquired from a single DNP sample.

Methanol in DNP NMR

Figure 1 shows a direct comparison of water and methanol as dissolution solvents, using identical samples and dissolution conditions. In this example, the observed signal-to-noise enhancement in methanol is approximately 33% larger than in water. This is partly a consequence of the improved flow properties of methanol leading to faster transfer, and partly of the longer T₁ values that this sample exhibits in methanol.

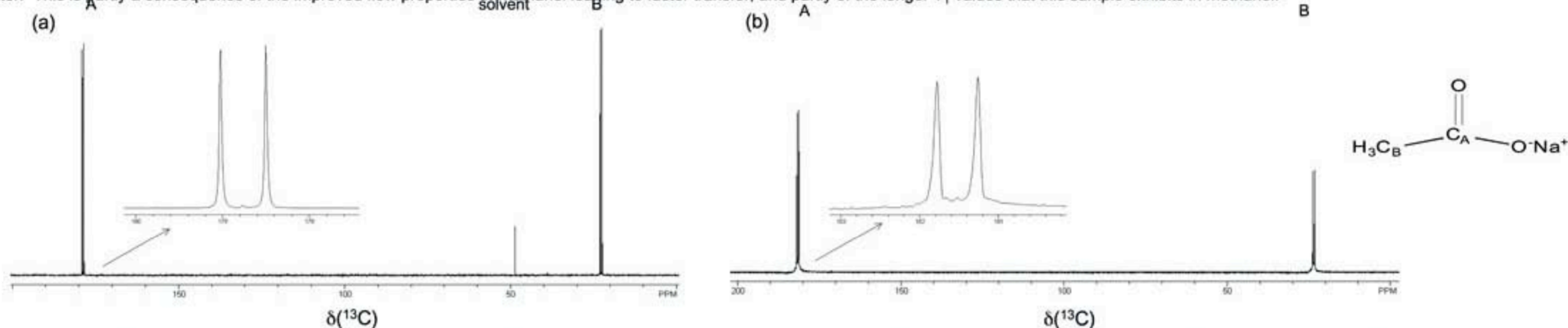


Figure 1. One-scan ¹³C DNP NMR spectra of ¹³C₂-sodium acetate (3.2 μmol in 20 μL of H₂O:DMSO 1:1), polarised for 1 h and dissolved under identical conditions in (a) methanol and (b) water. The vertical scale is constant in the main spectra and in the insets.

A more interesting application of methanol is to hyperpolarise molecules that are hydrophobic or otherwise incompatible with water. This is illustrated in Figure 2 for diphenylacetylene and benzophenone. Both spectra show excellent line shape (line width <3 Hz at half-height). The signal-to-noise enhancement based on a single scan¹ ranges from 500 (resonance C) to 2500 (resonance A) for diphenylacetylene and from 250 (resonance B) to 1000 (resonance E) for benzophenone.

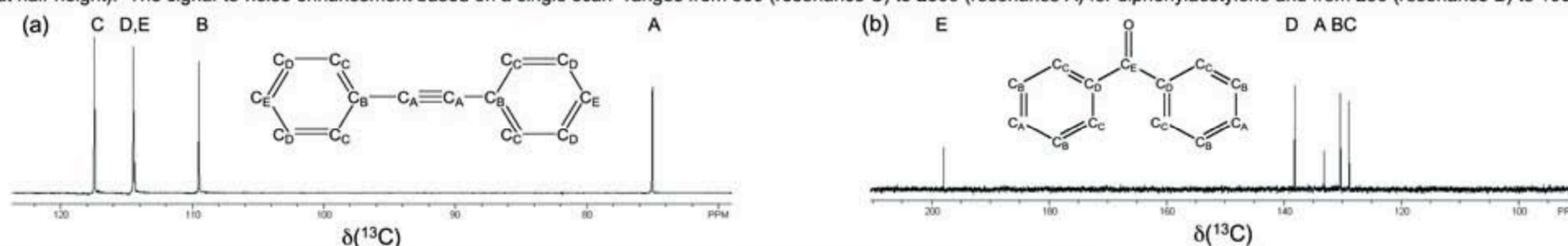


Figure 2. ¹³C DNP NMR spectra of natural abundance (a) diphenylacetylene (20 μmol) and (b) benzophenone (15 μmol), dissolved with 4 mL methanol after 2 hours of polarisation.

Detection of minor isomers

The additional signal-to-noise that can be obtained from methanol dissolutions further facilitates the use of ¹³C DNP NMR to detect minor components in a sample. An elegant demonstration is by reference to keto-enol tautomerism, as shown in Figure 3 for ethyl acetoacetate. The two spectra correspond to a single-scan DNP NMR measurement after three hours of polarisation and a conventional, thermal NMR spectrum with three hours of signal averaging. Identical sample amounts were used in each case. Crucially, the enol form is observed by ¹³C DNP NMR, but not detected by normal ¹³C NMR under these conditions. This demonstrates the ability of DNP NMR to detect the presence of a minor component in a sample, and thus potentially a minor constituent of a mixture, more readily than a comparable conventional NMR measurement.

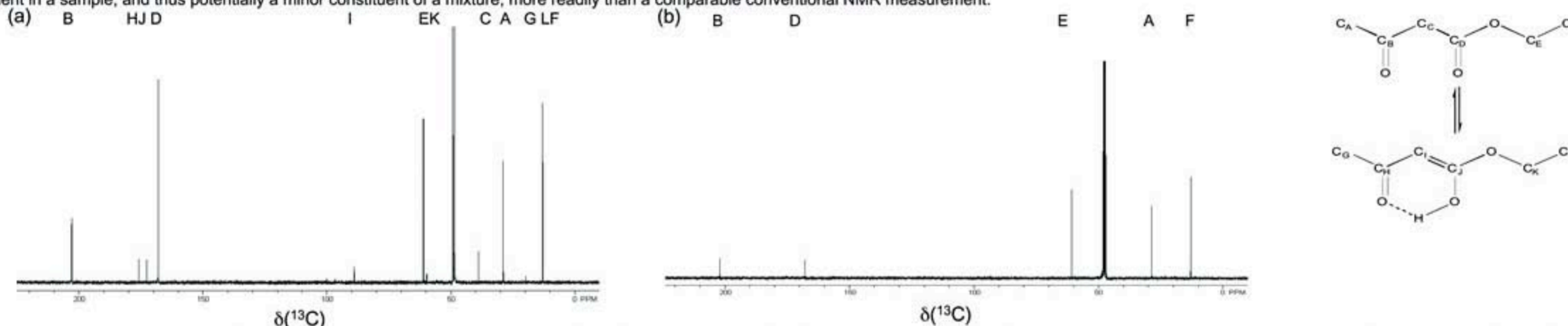


Figure 3. ¹³C DNP NMR spectra of natural abundance ethyl acetoacetate (60 μmol). Trace (a) corresponds to a one-scan DNP NMR spectrum after 3 hours polarisation and dissolution in 4 mL methanol, whilst trace (b) is a conventional NMR spectrum recorded over 3 hours in methanol-d₄. The vertical scale is identical in both spectra. The resonance at ca. δ 50 is due to the methanol solvent.

Polarisation transfer and multiple scans

Figure 4 illustrates a ¹³C DNP NMR experiment where a 1:1:1 mixture of 1-¹³C, 2-¹³C and 1,2-¹³C₂ sodium acetate was polarised and dissolved in water. The various ¹³C sites on the isotopomers were shown to polarise to the same extent in the solid state. The ratio of the carbonyl resonances for the two isotopomers was an unexpected 1.6:1 (1-¹³C:1,2-¹³C₂) despite their T₁ relaxation times being nearly identical (32.7 s and 33.1 s, respectively). The ratio of the methyl resonances also did not match expectations (1-¹³C:1,2-¹³C₂ = 0.8:1). Once again, the T₁ relaxation times of the two sites are nearly identical (9.2s and 9.4s, respectively). It is suggested that during sample transfer through the low magnetic field outside the two magnets, polarisation transfer can take place in the 1,2-¹³C₂ isomer from the slow relaxing carbonyl to the fast relaxing methyl (see Figure 4), lowering the intensity of the former and raising the intensity of the latter. In singly labelled isotopomers, the chance of two NMR active nuclei being together is much lower, and this effect is present to a lesser extent, accounting for the differences in intensity.

Another interesting observation is that, contrary to theoretical predictions, DNP NMR is not limited to just one scan. Eight consecutive scans obtained with 90° pulses after a dissolution with water are shown in Figure 5, and >150 spectra can be obtained with 30° pulses. This may be due to polarisation transfer (e.g. from associated protons) or to diffusion in the NMR tube causing fresh magnetisation to enter the coil region after the initial pulse is applied.

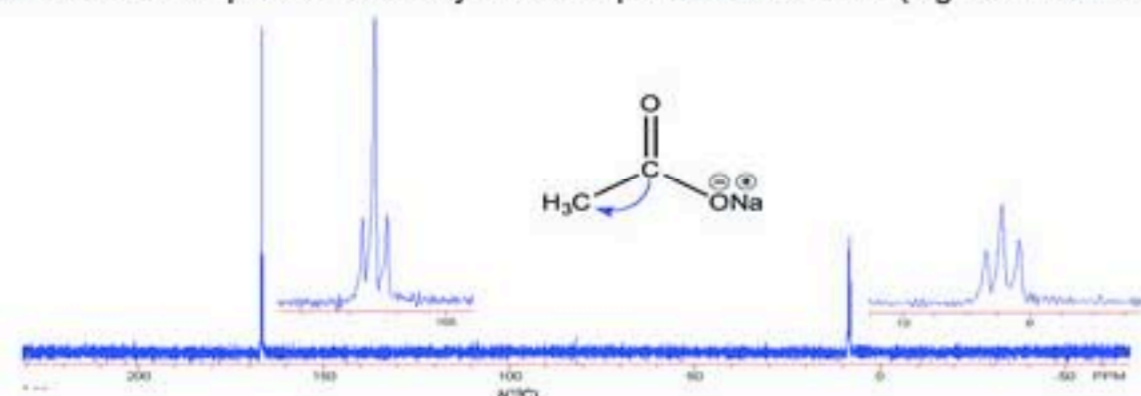


Figure 4. ¹³C DNP NMR spectrum of a 1:1:1 mixture of 1-¹³C, 2-¹³C and 1,2-¹³C₂ sodium acetate, dissolved in water. The ratios of the carbonyl and methyl resonances do not match expectations.

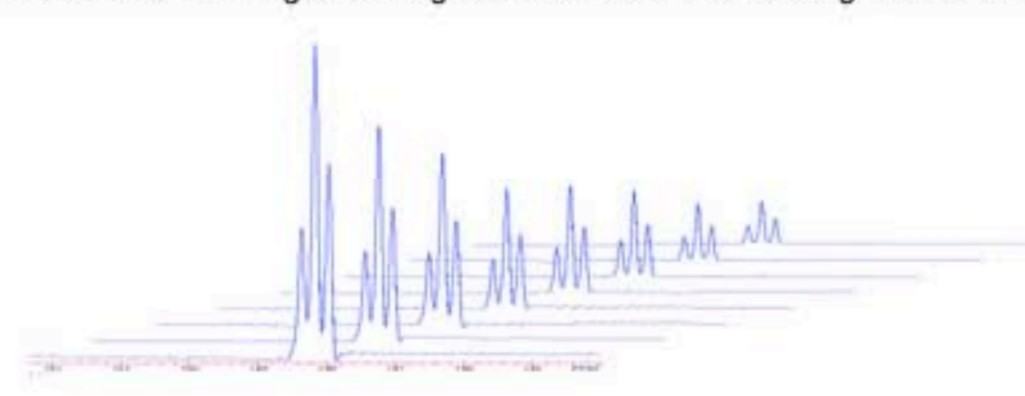


Figure 5. ¹³C DNP spectrum of ¹³C-¹⁵N-urea dissolved in water, showing eight consecutive transients acquired with 90° excitation pulses.

References

- Ardenkjaer-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M.H.; Servin, R.; Thaning, M.; Golman, K. *Proc. Nat. Acad. Sci.* **2003**, *100*, 10158.
- Wolber, J.; Ellner, F.; Fridlund, B.; Gram, A.; Johannesson, H.; Hansson, G.; Hansson, L.H.; Lerche, M.H.; Mansson, S.; Servin, R.; Thaning, M.; Golman, K.; Ardenkjaer-Larsen, J. H. *Nucl. Inst. Meth. Phys. Res. A* **2004**, *526*, 173.