



DNP NMR Dissolutions with Methanol

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- DNP NMR yields greatly enhanced resonances in solution-state NMR spectra, overcoming the poor sensitivity of this very versatile analytical technique. This makes it possible to directly detect low-abundance nuclei such as ^{13}C and ^{15}N in only one scan and use ^{13}C NMR in the currently unusual situation of detecting minor components in mixtures.
- Using methanol as the dissolution solvent in DNP NMR enables the user to bring the benefits of this approach to some hydrophobic samples, or those that are incompatible with water. In addition, the flow properties of methanol lead to faster sample transfer, improved spectral line shape and better signal-to-noise.

Introduction

To apply the benefits of Dynamic Nuclear Polarisation (DNP) to NMR spectroscopy, the sample of interest must be exposed to a magnetic field at very low temperature ($< 4\text{ K}$) in the presence of a trityl radical and a glassing agent¹. Under such conditions, the free electrons on the radical will become hyperpolarised. This hyperpolarisation can be transferred to atomic nuclei by microwave irradiation at an appropriate frequency. If the hyperpolarised sample is rapidly dissolved in a hot solvent and transferred into the magnet of a high field

NMR spectrometer, greatly enhanced resonances can be observed in resulting solution-state NMR spectra.

The HyperSense™ instrument is an automated, stand-alone polariser that enables users to easily exploit the benefits of DNP NMR. It is compatible with two dissolution solvents: water and methanol². The use of water as the dissolution solvent has been described previously^{1,3}. However, methanol is an interesting alternative for a number of applications.

Experimental considerations

As one of the simplest organic solvents, methanol can dissolve a number of hydrophilic and hydrophobic compounds. Its thermal properties, namely a heat capacity of $82\text{ J K}^{-1}\text{ mol}^{-1}$ and a liquid state that extends from -98 to $65\text{ }^\circ\text{C}$ at atmospheric pressure, make it suitable for DNP dissolutions. Typically, methanol will reach dissolution conditions at a temperature of $140\text{ }^\circ\text{C}$ and a pressure of 9 bar. It will strike the sample at a temperature of $72\text{ }^\circ\text{C}$ and will enter the NMR spectrometer at approximately $20\text{ }^\circ\text{C}$, depending on sample volume. The low viscosity of methanol ($0.55 \times 10^{-3}\text{ kg m}^{-1}\text{ s}^{-1}$ at 298 K), combined with low surface tension, mean that it flows through the narrow HyperSense™ transfer tubing under annular flow conditions and is very reliable for filling both 10 mm and 5 mm NMR tubes. From an NMR perspective, methanol exhibits only a single ^{13}C resonance at ca. $\delta\ 50.0$, minimising the risk of spectral overlap with the sample. These physical properties make methanol a desirable dissolution solvent.

To use methanol on the HyperSense™ instrument, it is not necessary to modify any of the existing water-based dissolution methods. However, improvements can be made if desired. If required, methanol dissolutions can be triggered as soon as the pressure in the dissolution vessel reaches 8 bar. Furthermore, the better flow properties of methanol mean that, on average, it takes one second less time to fill the NMR tube. The gas chase time can be therefore be decreased by one second and NMR measurement can start one second earlier, reducing signal losses due to T_1 relaxation.

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Dissolutions with methanol

Figure 1 shows a direct comparison of water and methanol as dissolution solvents. Two identical samples of $^{13}\text{C}_2$ -sodium acetate (3.2 μmol of material in 20 μL of $\text{H}_2\text{O}:\text{DMSO}$ 1:1) were polarised under identical conditions and dissolved into 5 mm NMR tubes using 4 mL of the respective solvent. All dissolution parameters were kept identical, and one-scan ^{13}C NMR spectra were acquired on a JEOL ECA-400 spectrometer using a 45° detection pulse. 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 T_1 values that this sample exhibits in methanol.

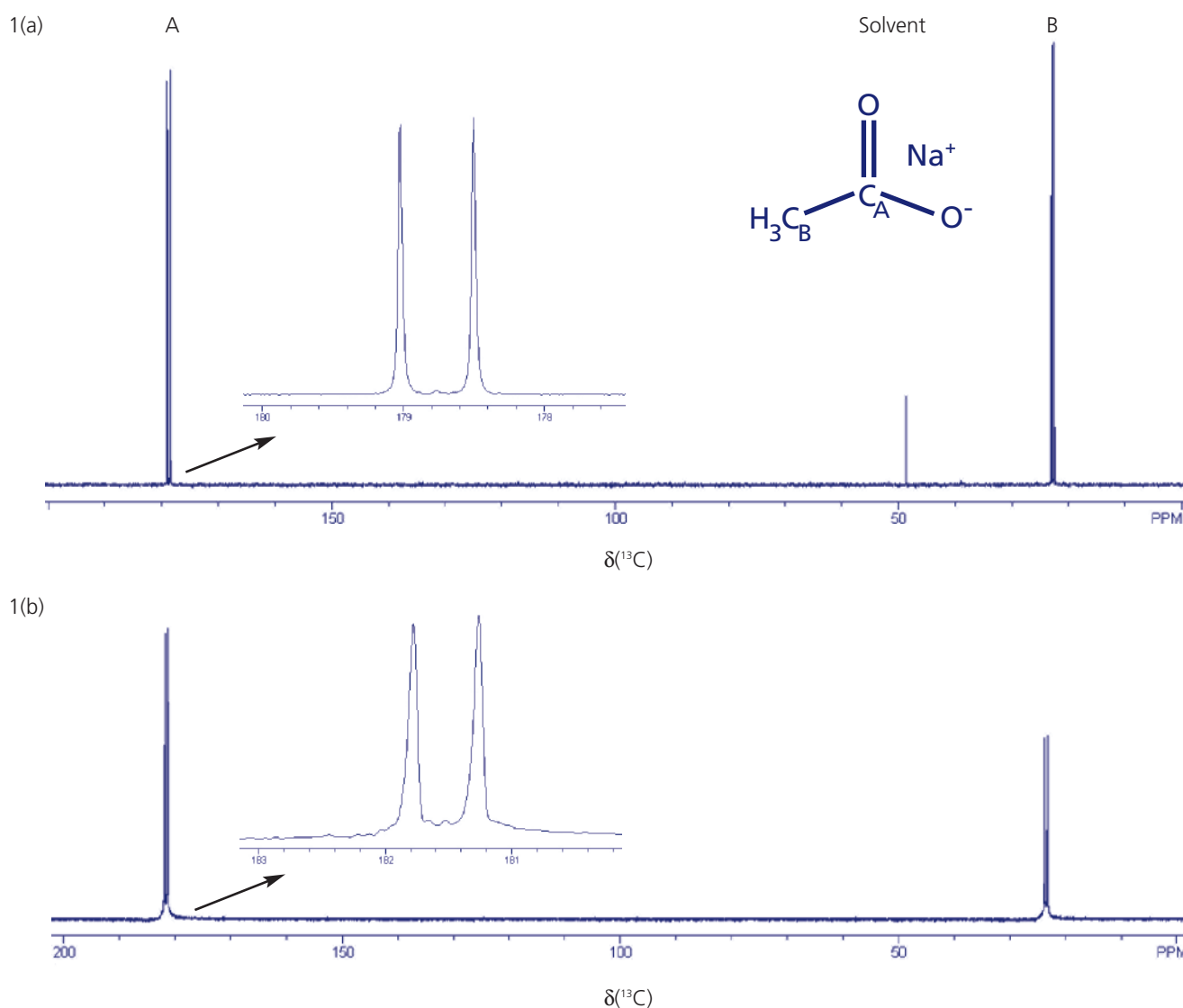


Figure 1. One-scan ^{13}C DNP NMR spectra of $^{13}\text{C}_2$ -sodium acetate (3.2 μmol in 20 μL of $\text{H}_2\text{O}:\text{DMSO}$ 1:1), polarised for 1 hour and dissolved under identical conditions in (a) methanol and (b) water. The vertical scale is constant in the two main spectra and in the two inserts.

A more interesting application of methanol is to hyperpolarise molecules that are hydrophobic or otherwise incompatible with water. Figure 2 shows the results obtained from hyperpolarised measurements on two such compounds, 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 scan ranges from 500-fold (resonance C) to 2500-fold (resonance A) for diphenylacetylene and from 250-fold (resonance B) to 1000-fold (resonance E) for benzophenone.

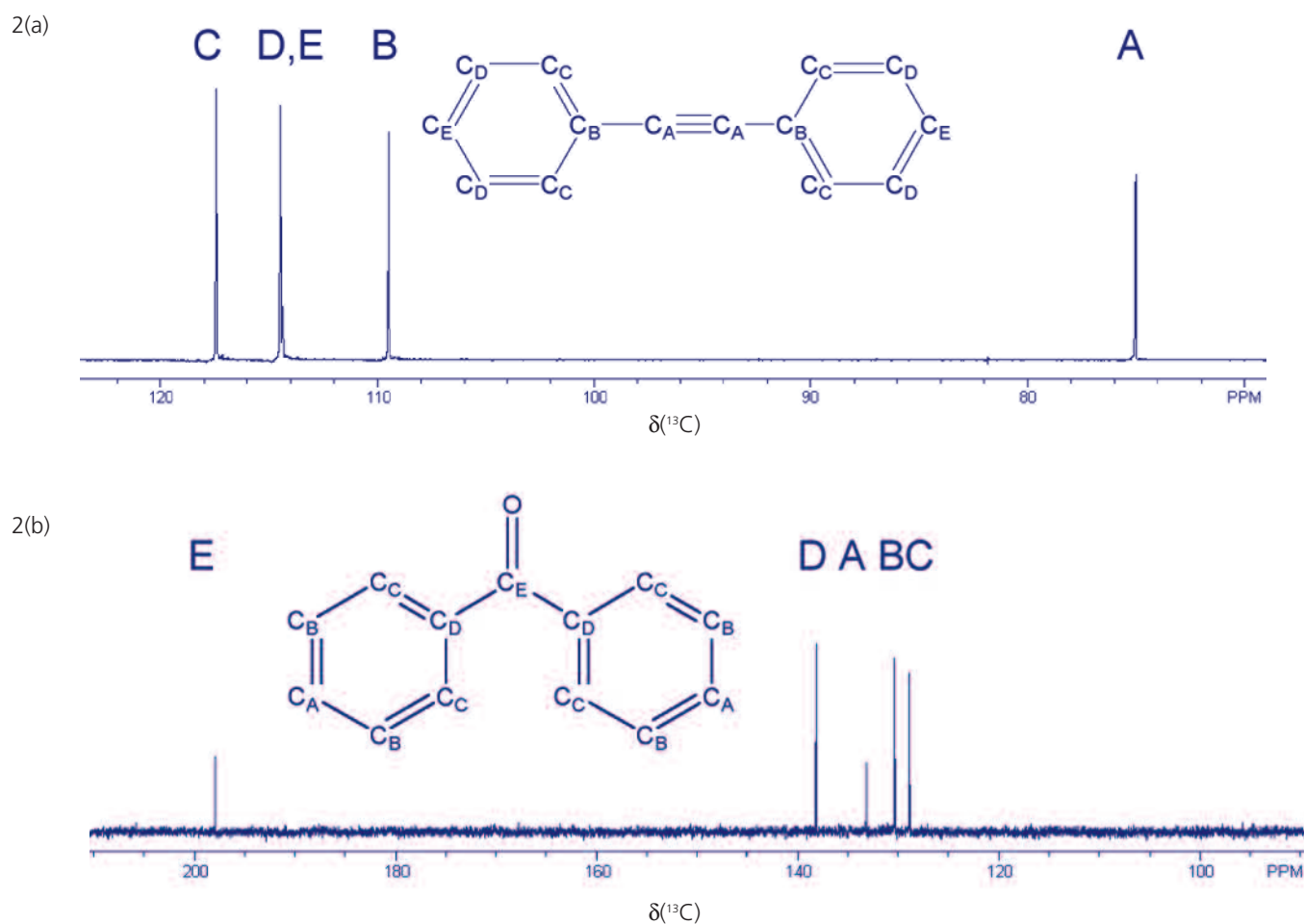


Figure 2. ^{13}C DNP NMR spectra of natural abundance (a) diphenylacetylene (20 μmol) and (b) benzophenone (15 μmol). Both compounds were dissolved with 4 mL methanol after 3 hours of polarisation.

Detection of minor isomers

The additional signal-to-noise that is obtained from methanol dissolutions also facilitates the use of ^{13}C DNP NMR to detect minor components in mixtures. An elegant demonstration is by reference to keto-enol tautomerism, an equilibrium that is important in a number of chemical and biochemical reactions, including the metabolism of glucose in the human body.

Figure 3 demonstrates the use of DNP NMR to study the keto-enol tautomerism of ethyl acetoacetate. This compound is of interest to the biochemical and chemical industries as it is an intermediate in the production of amino acids, analgesics and antibiotics, as well as in the manufacture of dyes and inks. The two spectra shown in Figure 3 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. The single-scan signal to noise enhancement observed for the keto isomer varies from 500-fold for resonance F to 3000-fold for resonance D. Crucially, the enol form is observed by ^{13}C DNP NMR, but not detected by normal ^{13}C 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 measurement.

Conclusions

The above experiments demonstrate some of the benefits that using methanol as the dissolution solvent can bring to DNP NMR spectroscopy. These include faster sample transfer, improved resolution and signal-to-noise, as well as studying molecules that are incompatible with water. It also facilitates the use of ^{13}C NMR in the currently unusual situation of detecting minor isomers, and potentially minor components in mixtures.

Hints and tips

- Methanol flows through HyperSense™ transfer tubing more quickly than water, so that the transfer delay can be made 1 second shorter. This may be of interest for samples with short T_1 values.

- Samples dissolved in methanol- d_4 can have longer T_1 values than those in methanol. The deuterated solvent is also compatible with HyperSense™.²

- When switching between dissolution solvents, it is very important to thoroughly clean and dry the dissolution assembly. The recommended procedure is two washes with the last solvent that was used, followed by two washes with the solvent that will be used next.

References

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3. 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.

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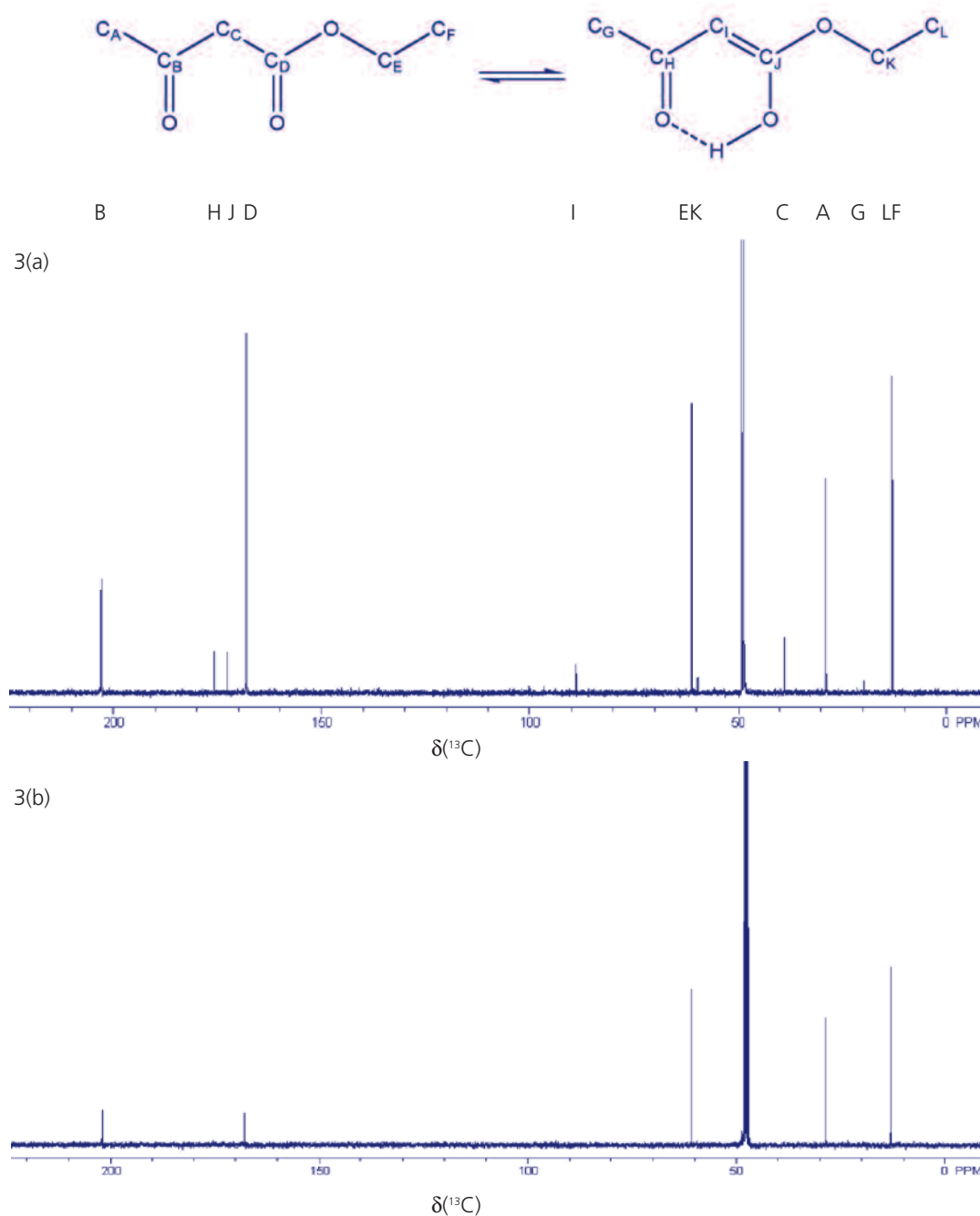


Figure 3. ^{13}C DNP NMR spectra of natural abundance ethyl acetoacetate (60 μmol). Trace (a) corresponds to a one-scan spectrum after 3 hours polarisation and dissolution in 4 mL methanol, whilst trace (b) is the thermal spectrum recorded over 3 hours in methanol- d_4 . The resonance at δ 50 is due to the methanol solvent.