



Influence of Trityl Radical on the DNP Process

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A key component in Dynamic Nuclear Polarisation is the trityl radical, which acts as the source of unpaired electrons. The polarisation process depends on the interactions between the radical and the molecules of interest, making it important to choose an appropriate radical for each sample.

Introduction

Dynamic Nuclear Polarisation (DNP) yields greatly enhanced signals for ^{13}C and ^{15}N nuclei in solution-state NMR spectroscopy. The technique involves cooling a sample to $<4\text{ K}$ in a strong magnetic field ($B_0 = 3.35\text{ T}$) in the presence of a trityl radical.¹ Under such conditions, unpaired electrons become strongly polarised, and this polarisation can be transferred to nearby atomic nuclei using microwave irradiation. Once the polarisation has built up to a sufficient level, the sample is dissolved by the injection of an aliquot (typically 3–5 mL) of hot solvent and rapidly ($<1\text{ s}$) transferred to a conventional NMR spectrometer for measurement. This procedure has been shown to yield a signal-to-noise enhancement of over 10,000 times compared to a conventional NMR experiment.^{1,2}

An important part of the hyperpolarisation process is the interaction between the trityl radical and the sample molecules, which causes the latter's magnetisation to become hyperpolarised. To optimise the polarisation process, the radical needs to be matched appropriately to the sample, which is primarily based on polarity.

Experimental considerations

Two trityl radicals are supplied by Oxford Instruments Molecular Biotoools Ltd. for use in DNP: a more hydrophobic species ("Finland") and a more hydrophilic one ("OX63"). Their structures are shown in Figure 1.

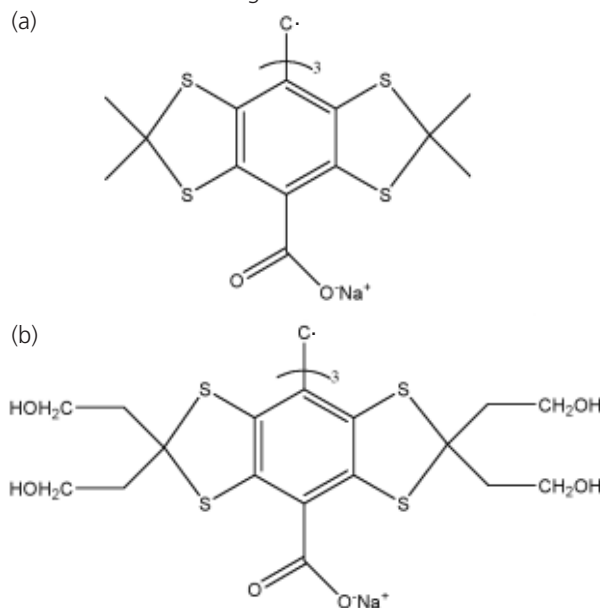


Figure 1. Structures of the trityl radicals: (a) "Finland"; (b) "OX63".

The examples that follow describe the effects of changing the radical on DNP NMR spectra. In each study, all polarisation and dissolution parameters were kept identical except for the type of the radical. Solid-state experiments were carried out on a DNP test-bed using a high-sensitivity solid-state diagnostic probe.³ Samples for solution-state experiments were polarised on a HyperSense™ instrument and dissolved prior to introduction into a JEOL ECA 400 MHz spectrometer equipped with a 5 mm broadband observe (BBO) probe. A single-scan spectrum using a 90° pulse was acquired in each case with inverse-gated decoupling.

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Solid-state performance of the trityl radicals

The parameters of interest in the solid state are the microwave frequency that will yield the maximum polarisation, the absolute polarisation magnitude and the time required to reach the maximum polarisation for each radical. The sample employed was a 2.0 M solution of ^{13}C - $^{15}\text{N}_2$ -urea.

The procedure used to determine the optimum microwave frequency for hyperpolarising each sample is called a "microwave sweep"³. Measurements were made between microwave frequencies of 93.90 and 94.10 GHz in steps of 5 MHz. At each frequency, the sample was irradiated for 10 minutes and the resulting magnetisation measured using a 90° pulse. Any residual magnetisation was then destroyed using a saturation sequence before the microwave frequency was changed. The free induction decay (FID) was apodised, zero-filled and Fourier transformed, and the integral of the resulting NMR signal was calculated. The NMR signal was converted to a polarisation estimate based on the thermal equilibrium signal obtained from the same sample. The data were also scaled to represent saturation polarisation for each sample (see below). The results obtained for ^{13}C and ^{15}N microwave sweeps for both radicals are shown in Figure 2.

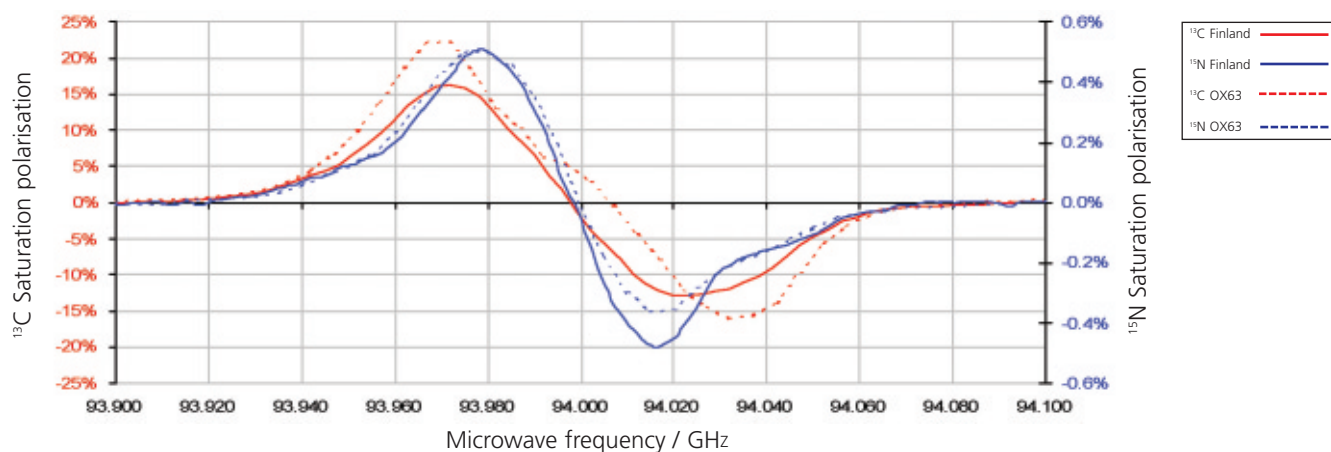


Figure 2. Microwave frequency dependence of saturation polarisation for ^{13}C and ^{15}N with the "Finland" and "OX63" radicals.

To study the evolution of hyperpolarisation with time, each sample was exposed to the lower frequency polarisation maximum at a temperature of 1.4 K. A simple pulse-acquire sequence was then used to apply a 5° pulse every 5 minutes. The results are shown in Figure 3.

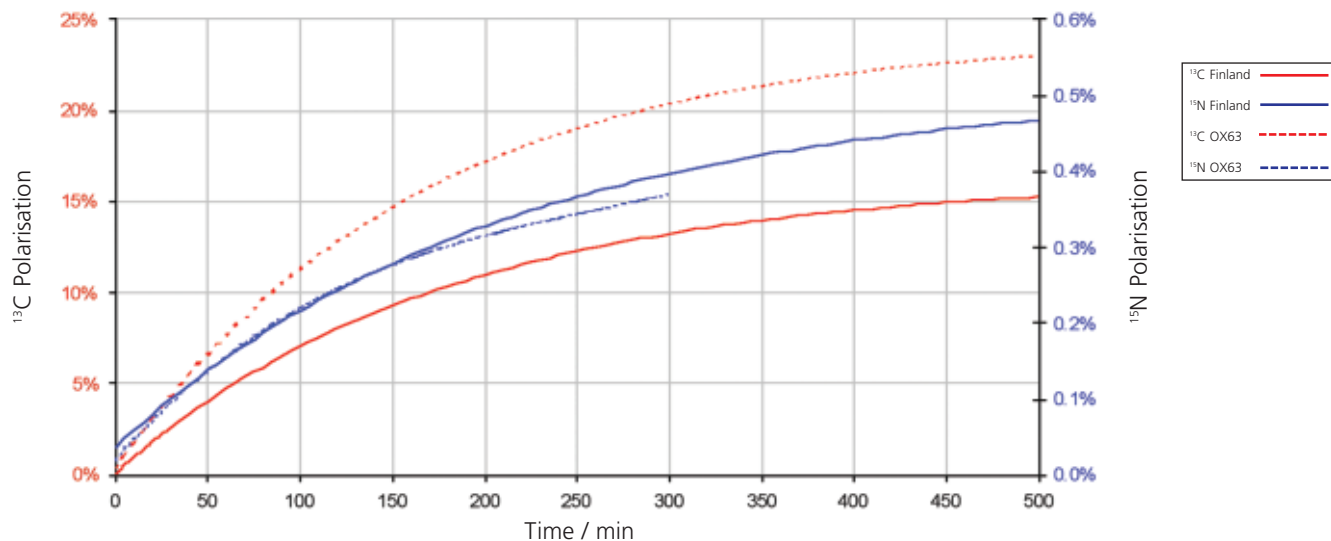


Figure 3. Time evolution of polarisation for ^{13}C and ^{15}N with the "Finland" and "OX63" radicals.

Both trityl radicals are clearly effective at hyperpolarising both ^{13}C and ^{15}N nuclei, and with both radicals ^{15}N seems to polarise to a lower extent than ^{13}C . However, the absolute extent of polarisation for ^{15}N should be treated as an estimate due to the difficulty in obtaining sufficient signal-to-noise in thermal solid-state ^{15}N spectra that were used for control (due to the small gyromagnetic ratio and the absence of magic angle spinning). In all four cases, the sweep curves are characteristic of a dominant thermal mixing DNP mechanism, although the microwave frequencies required for ^{13}C and ^{15}N to reach maximum polarisation are not the same. However, the frequency is independent of the identity of the radical.

The above results also indicate that for ^{13}C nuclei in urea, the "OX63" radical offers faster polarisation build up rate and higher maximum polarisation than the "Finland" radical. In contrast, for ^{15}N nuclei, "Finland" offers superior performance to "OX63".

Effect of radical on solution-state DNP NMR

The effect that the identity of the radical has on the DNP process varies with different sample molecules. In many cases, one of the radicals simply yields greater enhancement for all resonances; sometimes, one radical will only give a partial spectrum, whilst the other yields a full one; in other cases, one radical will give no polarisation at all.

An example where one trityl radical gives greater enhancement for all resonances is sodium acetate (Figure 4). In this case, the hydrophilic "OX63" radical is superior to the "Finland" radical.

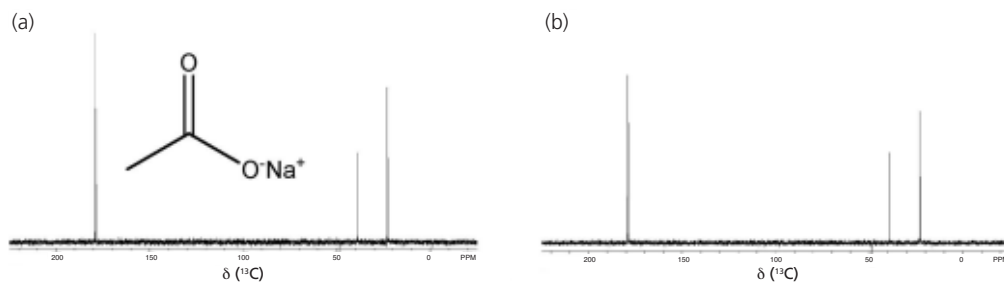


Figure 4. Single-scan ^{13}C DNP NMR spectra of sodium acetate in DMSO:water (1:1) at constant vertical scale, recorded after 1h polarisation with: (a) "OX63" radical; (b) "Finland" radical.

In the case of BOC-glycine, the "OX63" radical gives only a partial spectrum, whereas the "Finland" radical hyperpolarises all the resonances. This is illustrated in Figure 5.

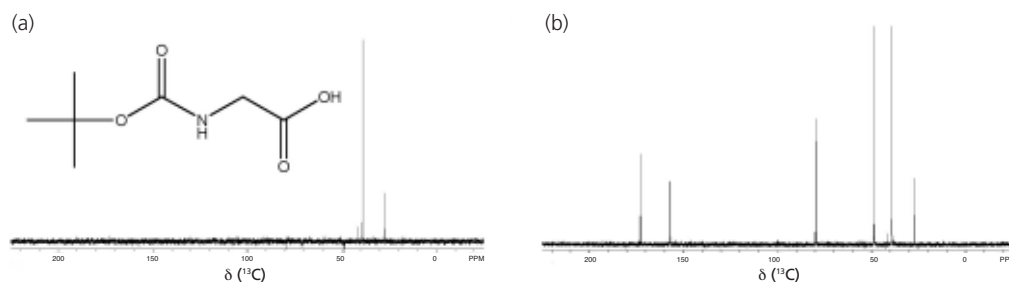


Figure 5. Single-scan ^{13}C DNP NMR spectra of BOC-glycine in methanol:DMSO (1:1), recorded after 3h polarisation with: (a) "OX63" radical; (b) "Finland" radical.

More extreme examples of the effect of the radical are provided by 1,2-Dimyristoil-sn-glycero-3-phosphocholine (DMPC), shown in Figure 6, and Leu-enkephalin acetate (Tyr-Gly-Gly-Phe-Leu), shown in Figure 7. The non-polar DMPC molecule is polarised effectively by "Finland", but not at all by "OX63"; in contrast, the polar Leu-enkephalin acetate is polarised by "OX63", but not by the "Finland" radical.

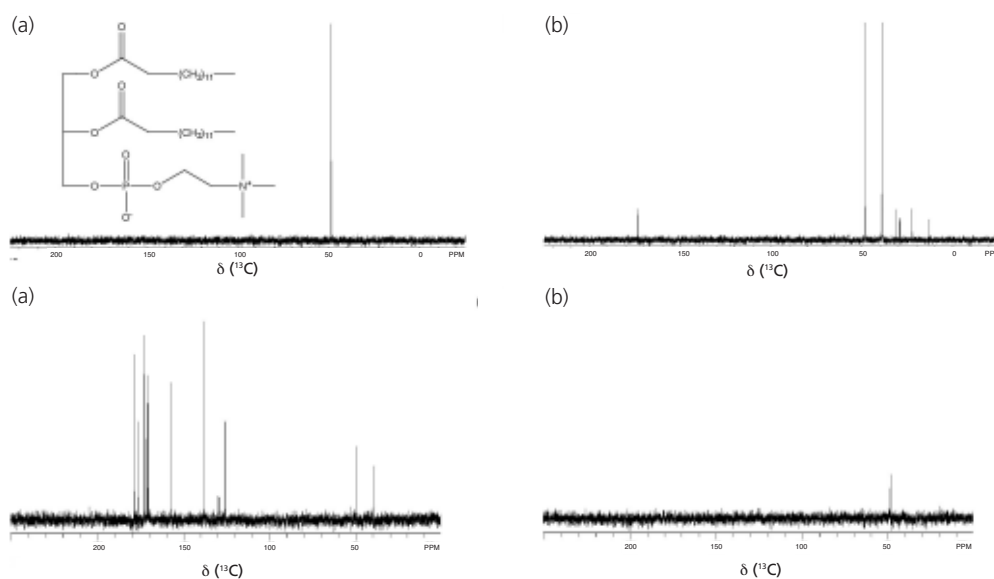


Figure 6. Single-scan ^{13}C DNP NMR spectra of DMPC polarised in methanol: DMSO (1:1) for 3h with: (a) "OX63" radical; (b) "Finland" radical.

Figure 7. Single-scan ^{13}C DNP NMR spectra of Leu-enkephalin acetate (Tyr-Gly-Gly-Phe-Leu) polarised in methanol:DMSO (1:1) for 3h with: (a) "OX63" radical; (b) "Finland" radical.

Polarising mixtures

The choice of radical is particularly important when mixtures of compounds are to be hyperpolarised. This is illustrated in Figure 8 for a mixture of DMPC and Leu-enkephalin acetate, two compounds of very different polarity. Two samples were used in this study. The first (Sample 1) consisted of the two compounds and a mixture of the two radicals, each present at 15 mM concentration, and was polarised for 4 hours. The main component of the resultant spectrum (Figure 8a) is Leu-enkephalin, although minor peaks attributed to DMPC can also be seen at δ 28.8, 28.4 and 21.8. It should be noted that Mixture 1 contains a total radical concentration of 30 mM, and that both radicals are randomly distributed through the sample. It is known that the polarisation process employed in this study (thermal mixing) depends crucially on the physical separation between the radical and the compound of interest.⁴ Since the

components in the mixture require different radicals for optimum polarisation, it is possible that the peak intensity in Sample 1 is low due to unsuitable separation between each molecule and its preferred radical. Furthermore, it is known that high radical concentrations ultimately give lower maximum polarisation, such that a radical at 30 mM leads to a relative loss of 25% in signal intensity compared to a radical at 15 mM.³

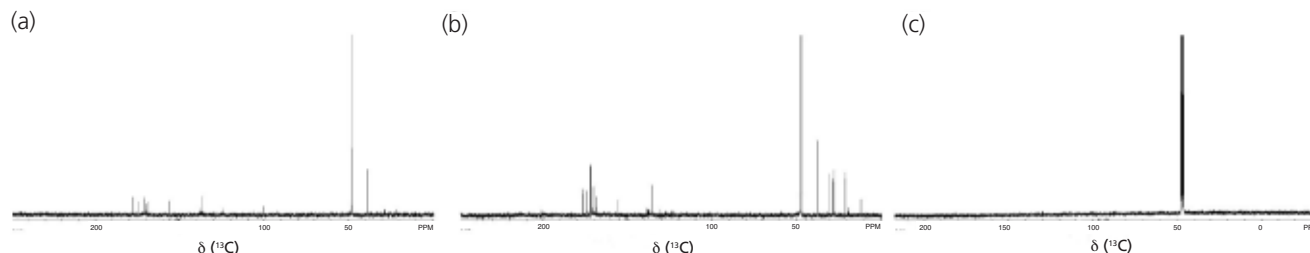


Figure 8. ¹³C NMR spectra of mixtures of DMPC and Leu-enkephalin acetate: (a) One-scan hyperpolarised spectrum of homogeneous Sample 1; (b) One-scan hyperpolarised spectrum of layered Sample 2; (c) Conventional spectrum, 16,000 scans.

To further test the effects of these parameters, Sample 2 was prepared, consisting of two layered components: the first layer contained DMPC and the “Finland” radical; the second layer contained Leu-enkephalin acetate and the “OX63” radical. Each layer was flash-frozen before being introduced into the polariser to prevent mixing. The radical concentration in each layer (and therefore the overall concentration in the sample) was 15 mM, which was expected to increase the maximum polarisation compared to Sample 1. The ¹³C DNP NMR spectrum of this sample obtained after 4 hours of polarisation is shown in Figure 8b. The spectrum is clearly superior to Sample 1, containing carbonyl and aromatic peaks corresponding to Leu-enkephalin acetate at improved signal-to-noise (in the regions δ 178-170 and δ 157-125, respectively) as well as the carbonyl and aliphatic chain peaks of DMPC. Mixtures of polar and non-polar samples can therefore be polarised by appropriate sample preparation, and significant signal enhancement can be obtained over conventional NMR spectroscopy (see Figure 8c).

Conclusions

The above experiments demonstrate the effects of different trityl radicals on the DNP process. The radical does not affect the microwave frequency that yields maximum sample polarisation, but it does influence the absolute polarisation level, both in solution and in the solid state. Therefore, to maximise the benefits of DNP to each sample, it is important to choose the most appropriate radical, generally based on the polarity of the sample. Further investigations will be carried out in this area.

Hints and tips

- Both radicals have the same optimal microwave irradiation frequency for a given sample. Therefore, if the only change to a sample is the radical, it is not necessary to repeat the microwave sweep.
- In general, the polarity of the molecule is the most important parameter when trying to predict the most appropriate radical for a new sample: “Finland” is usually better for non-polar molecules and “OX63” is better for polar molecules. There are some exceptions, for example glucose polarises better with “Finland”.
- The above feature can be used to selectively polarise a part of a mixture. For example, suppose that a sample is saturated with glucose, but also contains several minor components at 1% level. If the amount of glucose in the sample is of interest, the sample should be polarised with “Finland”. This will enhance the glucose peak, but due to dynamic range issues, other components may be difficult to detect. If, on the other hand, the minor components are of interest, the sample should be polarised with “OX63”, which will not affect glucose and hence yield a spectrum that will be free from large glucose resonances.
- For a more detailed account of the effects that the concentration of the trityl radical can have on the extent of polarisation, please see the HyperSense™ application note “*Characterising Solid-State DNP*”.³
- Both the “Finland” and the “OX63” radical can be purchased from Oxford Instruments Molecular Biotoools Ltd.

References

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