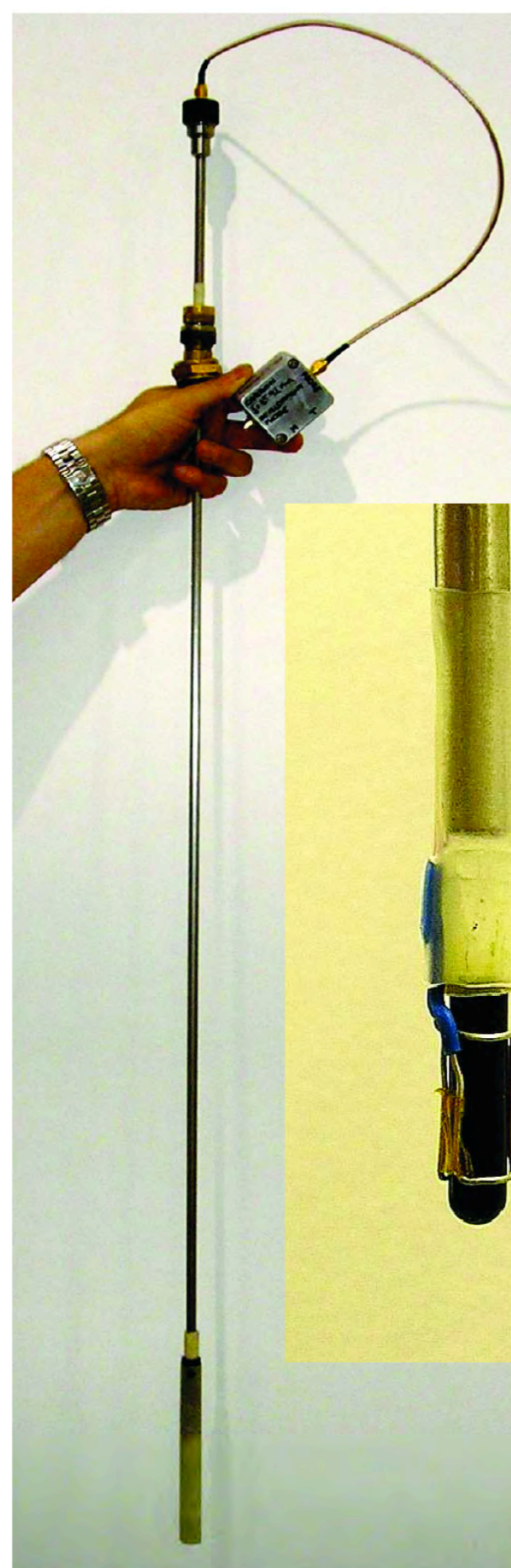


### Introduction

Dynamic nuclear polarisation (DNP) yields greatly enhanced signal intensity for  $^{13}\text{C}$  and  $^{15}\text{N}$  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.<sup>1</sup> Under such sample conditions, the unpaired electron on the trityl radical is strongly polarised, and this polarisation can be transferred to nearby atomic nuclei using microwave irradiation. Subsequent thawing and rapid introduction of the sample into a high-field NMR spectrometer has been shown to yield a signal-to-noise enhancement of over 10,000 times compared to a conventional NMR experiment.<sup>1,2</sup> However, to maximise the benefits this technique offers, it is necessary to understand and optimise the solid-state polarisation process.

### Experimental considerations

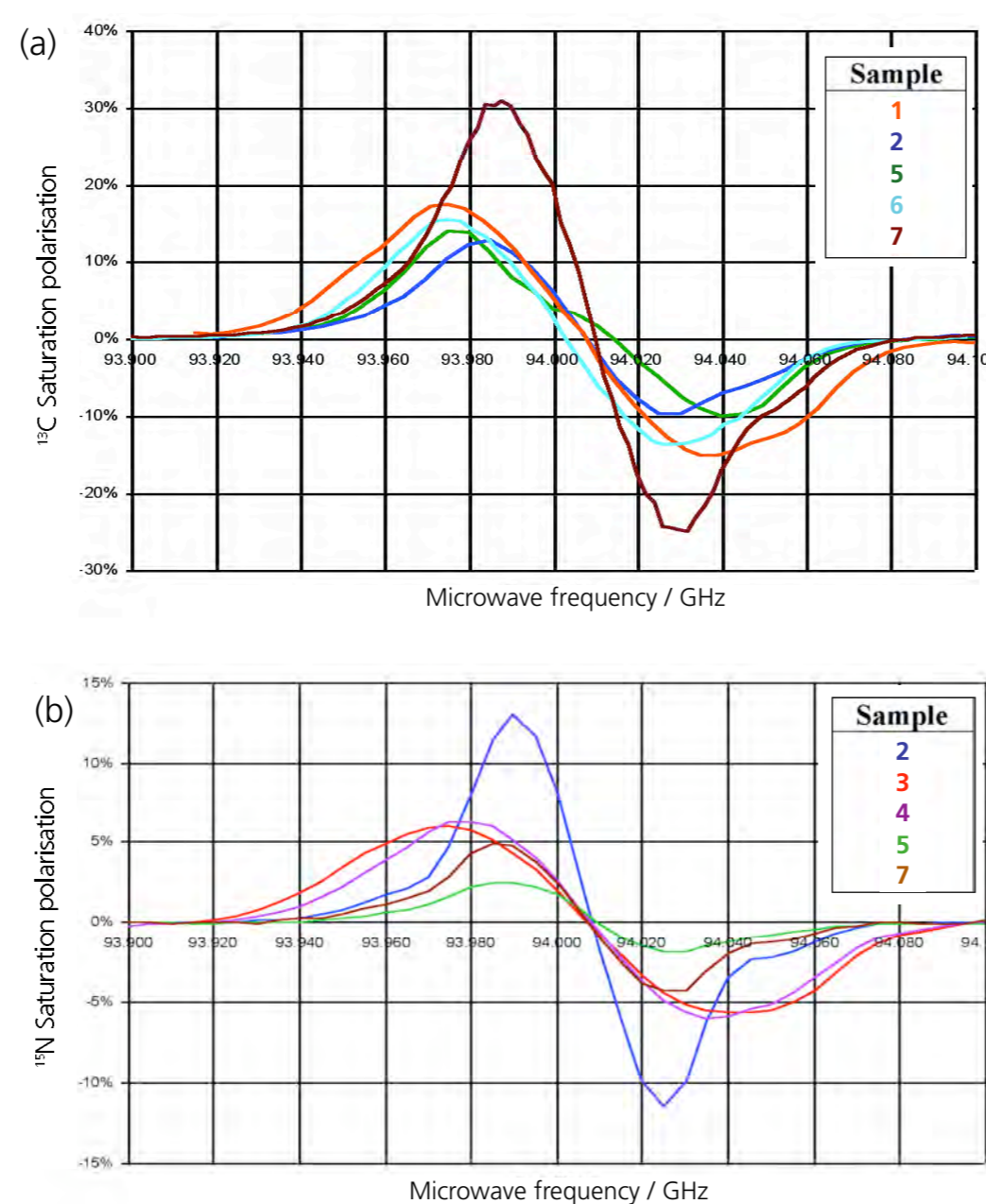
The tests described in this paper have been carried out on a DNP test-bed using a dedicated solid-state diagnostic probe (see Figure 1). This probe is placed into the polariser instead of the dissolution assembly. The filling factor of this probe has been optimised for solid-state research, giving improved  $^{13}\text{C}$  sensitivity. The probe can also be tuned to the  $^{15}\text{N}$  frequency. The samples that were used in the investigation are listed in Table 1.



**Figure 1.** The diagnostic solid-state research probe. The insert shows the coil and sample with the protective cover removed.

### Hyperpolarisation dependence on microwave frequency

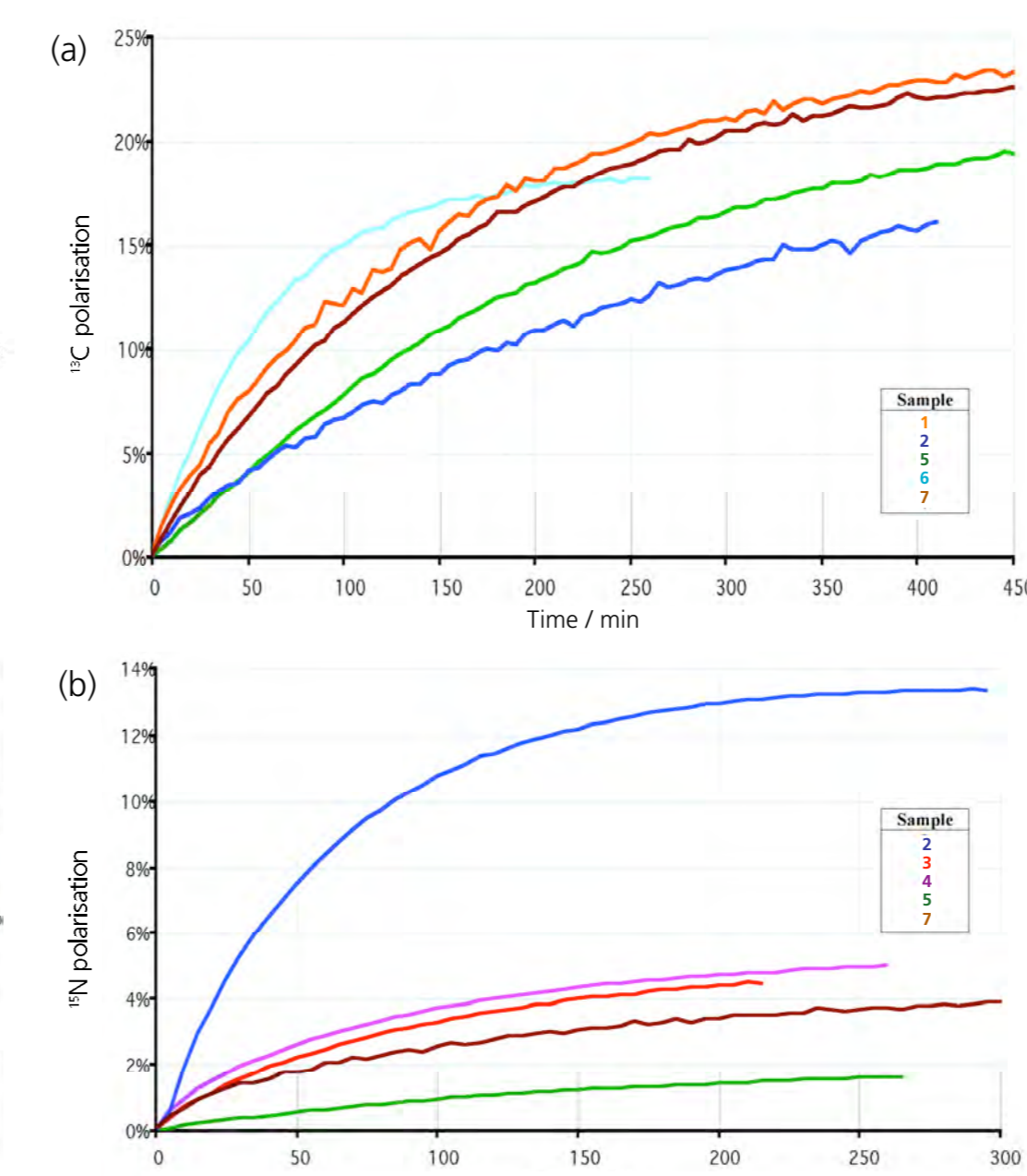
The procedure to determine the optimum microwave frequency for hyperpolarising each sample is called a "microwave sweep". Measurements were made between microwave frequencies of 93.90 and 94.10 GHz in steps of 5 MHz. At each frequency, the sample was irradiated for 10 minutes and the resulting magnetisation measured using a  $90^\circ$  pulse. Any residual magnetisation was destroyed using a saturation sequence. The FID was apodized, zero-filled and Fourier transformed, and the integral of the resulting NMR signal was calculated. The NMR signal was converted to a polarisation estimate using a calibration based on the sample's spin density in comparison to the thermal equilibrium signal obtained from a reference sample (sample #2 for  $^{15}\text{N}$  and #6 for  $^{13}\text{C}$ ). The data were also scaled to represent saturation polarisation for each sample (see below). The results obtained for both  $^{13}\text{C}$  and  $^{15}\text{N}$  microwave sweeps are shown in Figure 2.



**Figure 2.** Microwave frequency dependence of saturation polarisation for (a)  $^{13}\text{C}$  and (b)  $^{15}\text{N}$ .

**Table 1.** Sample compositions for solid-state DNP investigations. All samples also contain a trityl radical<sup>3</sup> at 15 mM concentration. The keys are used as identifiers in Figures 2 and 3.

Sample key	Composition	$^{13}\text{C}$ spin concentration / mol dm <sup>-3</sup>	$^{15}\text{N}$ spin concentration / mol dm <sup>-3</sup>
1	$^{13}\text{C}$ -1-alanine in water and ethylene glycol (1:1)	0.6	-
2	$^{15}\text{N}$ -pyridine and ethylene glycol (1:1)	0.3	6.2
3	$\text{Na}^{15}\text{NO}_3$ in water and ethylene glycol (1:1)	-	5.1
4	$\text{NH}_4^{15}\text{NO}_3$ in water and ethylene glycol (1:1)	-	5.4
5	$^{13}\text{C}$ - $^{15}\text{N}_2$ -urea in water and DMSO- $d_6$ (1:1)	3.0	5.9
6	$^{13}\text{C}$ -DMSO and water (1:1)	14.0	-
7	$^{13}\text{C}$ - $^{15}\text{N}_2$ -urea in $^{15}\text{N}$ -pyridine, water and DMSO- $d_6$	1.6	3.1



**Figure 3.** Time evolution of polarisation for (a)  $^{13}\text{C}$  and (b)  $^{15}\text{N}$ .

The enhancement curves are characteristic of a dominant thermal mixing DNP mechanism. Generally, they are quite similar in shape, but individual compounds show a difference in both the extent of saturation polarisation and the optimum polarisation frequency. It is interesting that the  $^{13}\text{C}$  compounds show a gradual spread in the optimum frequency, whereas with  $^{15}\text{N}$  there is a very distinct difference between organic and inorganic compounds. It should be noted that deuterated solvent mixtures can also be hyperpolarised.

### Time evolution of hyperpolarisation

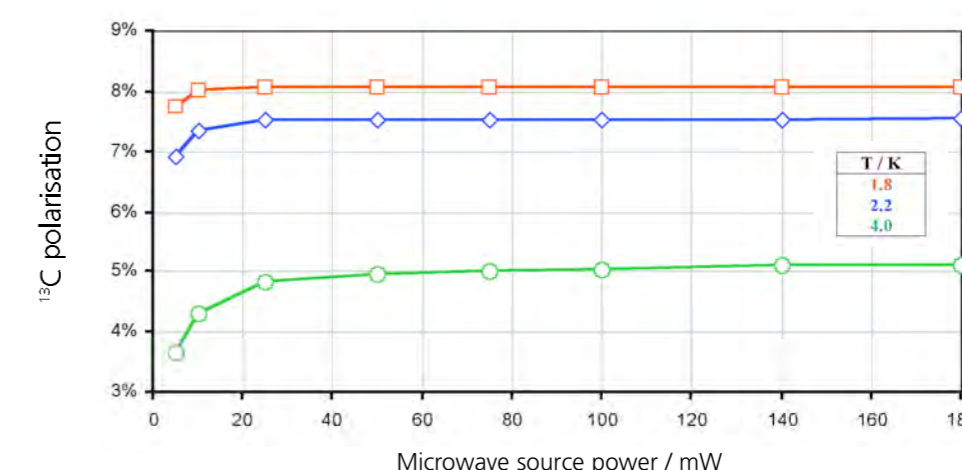
To study the evolution of hyperpolarisation with time, each sample was exposed to the lower frequency polarisation maximum at a temperature of 1.4 K. A simple pulse-acquire sequence was then used to apply a  $5^\circ$  pulse every 5 minutes. The results are shown in Figure 3. The trityl radical is clearly effective at hyperpolarising both  $^{13}\text{C}$  and  $^{15}\text{N}$ . There is a significant spread in both the build-up time and the saturation polarisation between different compounds, but in general  $^{15}\text{N}$  seems to polarise faster and to a lower extent than  $^{13}\text{C}$ .

### Influence of temperature, microwave power and trityl radical concentration

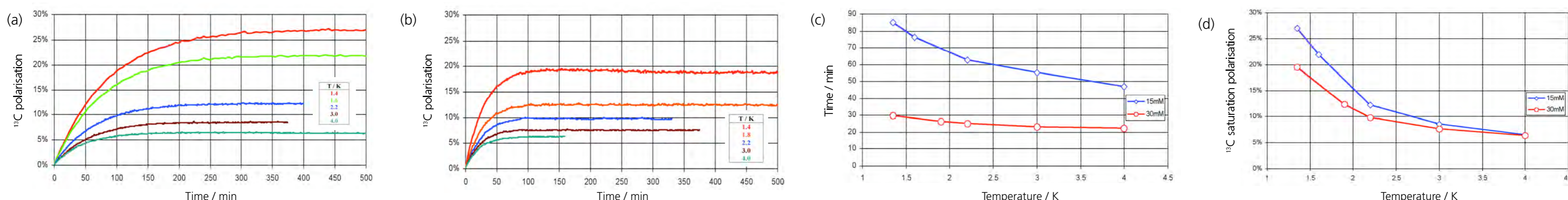
To study the effects of these parameters on the hyperpolarisation of  $^{13}\text{C}$ , sample #7 was utilised and a  $5^\circ$  pulse was applied every 5 minutes.

The effect of microwave source power on the saturation polarisation is illustrated in Figure 4. If the source power is above 60 mW, there is very little effect on the final polarisation reached. Below 60 mW the polarisation decreases, and the effect is more pronounced at higher temperatures. Since higher microwave power affects helium consumption, the optimum microwave power setting is in the region of 80 to 100 mW.

Figure 5 shows the effects of temperature on the time-evolution of polarisation at two radical concentrations. In both cases, there is a clear trend: both the saturation polarisation and the rate of polarisation build-up show an inverse dependence on temperature. At constant temperature, higher radical concentration leads to lower saturation polarisation, but faster build-up. Therefore, if only a short polarization time is available, it is advantageous to utilise a higher radical concentration. However, the lowest available temperature should be used at all times.



**Figure 4.** Dependence of  $^{13}\text{C}$  saturation polarisation on microwave source power.



**Figure 5.** (a) Time evolution of  $^{13}\text{C}$  polarisation build-up for sample #7 in the presence of 15 mM trityl radical; (b) As "a" with 30 mM trityl radical; (c) Temperature dependence of the  $^{13}\text{C}$  polarisation build-up exponential time constant, grouped by radical concentration; (d) Temperature dependence of the  $^{13}\text{C}$  saturation polarisation, grouped by radical concentration.

### Conclusions

The tests presented above highlight the importance of understanding and optimising the solid-state DNP process if maximum sensitivity increase is to be obtained from DNP NMR. The microwave frequency should be chosen according to the nucleus ( $^{13}\text{C}$  or  $^{15}\text{N}$ ) and sample of interest. The microwave source power should also be set appropriately, since low values lead to lower polarisation and high values can increase helium consumption. Sample temperature and radical concentration are also user-adjustable parameters and can be changed depending on the available time and desired extent of polarisation.

### References

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- The OX63 trityl radical, patented by GE Healthcare, was used in all experiments.