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In Situ Temperature Jump High-Frequency Dynamic Nuclear Polarization Experiments: Enhanced Sensitivity in Liquid-State NMR Spectroscopy

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Abstract: We describe an experiment, in situ temperature jump dynamic nuclear polarization (TJ-DNP), that is demonstrated to enhance sensitivity in liquid-state NMR experiments of low-γ spins — ^{13}C, ^{15}N, etc. The approach consists of polarizing a sample at low temperature using high-frequency (140 GHz) microwaves and a biradical polarizing agent and then melting it rapidly with a pulse of 10.6 μm infrared radiation, followed by observation of the NMR signal in the presence of decoupling. In the absence of polarization losses due to relaxation, the enhancement should be $\epsilon^{\dagger} = \epsilon(T_{obs}/T_{\mu wave})$, where $\epsilon^{\dagger}$ is the observed enhancement, ϵ is the enhancement obtained at the temperature where the polarization process occurs, and $T_{\mu wave}$ and T_{obs} are the polarization and observation temperatures, respectively. In a single experimental cycle, we observe room-temperature enhancements, $\epsilon^{\dagger}$, of ^{13}C signals in the range 120−400 when using a 140 GHz gyrotron microwave source, $T_{\mu wave} = 90$ K, and $T_{obs} = 300$ K. In addition, we demonstrate that the experiment can be recycled to perform signal averaging that is customary in contemporary NMR spectroscopy. Presently, the experiment is applicable to samples that can be repeatedly frozen and thawed. TJ-DNP could also serve as the initial polarization step in experiments designed for rapid acquisition of multidimensional spectra.

1. Introduction

The past decade has witnessed a renaissance in the development of approaches to prepare samples with high nuclear spin polarizations with the goal of increasing signal intensities in NMR spectra of solids and liquids. These approaches have included high-frequency, microwave-driven dynamic nuclear polarization (DNP),[1−9] para-hydrogen-induced polarization (PHIP),[10,11] polarization of noble gases such as He, Xe,[12−14] and more recently Kr,[15] and optically pumped nuclear polarization of semiconductors[16−18] and photosynthetic reaction centers and other proteins.[19−22] All of these approaches successfully yield highly polarized spins and are studied to elucidate features of the polarization processes or of the material being polarized. However, one of the most appealing aspects of high-polarization methods is the possibility of transferring the polarization from the source to a surrounding medium, such as a solvent, and subsequently distributing the polarization to chemically, physically, or biologically interesting solutes. For this to occur, it is necessary that the polarizing agent be strongly coupled to the lattice of nuclear spins, and in this regard paramagnetic polarizing agents are appealing since the large magnetic moment of the electron spin couples effectively to its surrounding nuclei.

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Accordingly, high-frequency microwave (≥140 GHz)-driven DNP experiments using stable free radicals as polarizing agents[2,3] are currently used successfully to polarize a variety of systems, including solid polymers,[4,23−27] frozen solutions of small molecules,[3] amino acids,[5,6] virus particles,[7] soluble and membrane proteins,[8] and amyloid nanocrystals,[9] achieving enhancements, ϵ, of ∼50−400, depending on the details of the experiment.

In addition to polarizing solid samples, there is considerable interest in using high-frequency DNP to enhance the sensitivity of liquid-state NMR experiments. However, the polarization mechanisms operative in dielectric solids at high fields — the solid effect,[28,29] the cross effect,[2,30] and thermal mixing[28] — are not applicable to liquids. Instead, the Overhauser effect (OE)[31,32] is the dominant polarization mechanism, and it is efficient only at low magnetic fields. In particular, for small molecules, the rotational or translational correlation times are $\sim 10^{-12}$ s, and at low magnetic fields the condition $\omega_s \tau_c \leq 1$ is satisfied (where ω_s is the electron Larmor frequency and τ_c the correlation time) and the Overhauser effect is effective in transferring polarization. However, in the high-field regime commonly employed in contemporary NMR experiments, ω_s is large, the rotational and translational spectral densities are vanishingly small, and the Overhauser enhancements decrease significantly.[33] Thus, to enhance the polarization of liquid samples in high-field experiments, an alternative method is required.

In this paper we explore one solution to this problem that leads to enhancements in the range of 120−400 in spectra of low-γ spins such as ^{13}C and ^{15}N. In particular, we polarize the ^{1}H spins in the sample at low temperatures (∼90 K) using low concentrations of biradical polarizing agents.[2,34] That polarization is transferred to low-γ spins with cross polarization, the sample is melted with an infrared laser pulse, and the enhanced signal is observed in the presence of ^{1}H decoupling. The entire cycle can be repeated in situ and signal averaging performed as is customary in contemporary NMR experiments. If the polarization step were performed at a lower temperature, then a larger enhancement factor would be observed. Because of the freezing and thawing processes, this version of the experiment will find greatest application to studies of systems that can be repeatedly frozen and thawed, such as small molecules, and to a field such as metabolic screening.

2. Experimental Section

Samples for the experiments consist of solutions containing high concentrations of ^{13}C-labeled small molecules to facilitate observation of signals in the absence of DNP. In particular, the high concentration facilitates observation of the signal intensity in the absence of microwave irradiation and therefore measurement of the enhancement. For example, in the experiments below we used 2 M ^{13}C-urea in 50% $^2H_6$-DMSO and 50% water (80% ^2H_2O/20% H_2O). The solution was prepared with 3−5 mM TOTAPOL[34] as the biradical polarizing agent. About 9 μL of sample was placed in a 2.5 mm o.d. quartz capillary, and NMR measurements were conducted in a custom-designed probe in a 5 T magnet (211 MHz for ^{1}H and 53.31 MHz for ^{13}C). Continuous microwave irradiation was generated with a 140 GHz gyrotron.[35] The sample was maintained at ∼90 K by circulating cold N_2 gas during the experimental cycle. Typically, the equilibrium polarization buildup required 15−40 s (the ^{1}H T_1 is typically 5−10 s), and the enhancement in the solid-state spectra, ϵ, was ∼290 at this temperature and magnetic field. The rapid temperature jump (TJ) was performed by irradiating the sample with 10.6 μm radiation from a CO_2 laser transmitted to the sample through a multimode hollow optic fiber. Haw and co-workers[36,37] used a similar approach in TJ experiments on polymers, with the exception that the sample was larger (5 mm diameter rotors) and required higher laser power. Thus, it was necessary to use lenses rather than an optic fiber to irradiate the sample. After melting, the solution NMR spectrum was recorded in the presence of decoupling, and the sample was refrozen and polarized again for another experimental cycle. With our current experimental apparatus, the freezing typically required 60−90 s and the melting <1 s. Figure 1 illustrates the cycle used in the TJ-DNP experiments — cooling, polarization with microwaves, melting with IR radiation, and observation of the liquid-state spectrum. Figure 2 shows the pulse sequence associated with these steps, and it incorporates storage/retrieval pulses prior to and following the melting step of the experiment.

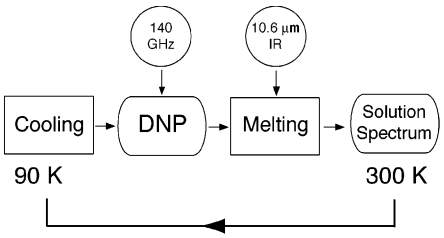

Figure 1. TJ-DNP cycle consisting of cooling, polarization, melting, and acquisition employed in the experiments described here. The microwaves for the DNP process were supplied by a 140 GHz gyrotron,[22] and the melting was accomplished with a 10.6 μm CO_2 laser.[36,37] With the current configuration of the apparatus, the experiment can be recycled every 60−90 s.

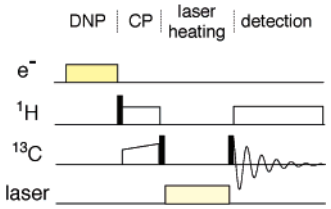

Figure 2. Experimental scheme for observation of sensitivity-enhanced liquid-state NMR using temperature jump DNP. The samples are irradiated with 140 GHz microwaves at ∼90 K, polarizing the ^{1}H spins in the sample. Enhanced ^{1}H polarization is then transferred to ^{13}C via cross polarization. During the laser heating, the ^{13}C magnetization is stored along the *z*-axis of the rotating frame. The ^{13}C spectrum is detected following a 90° pulse in the presence of WALTZ ^{1}H decoupling.

Enhancements, $\epsilon^\dagger$ (vide infra), were determined by comparing the signal intensities of the DNP-enhanced ^{13}C signal intensities obtained

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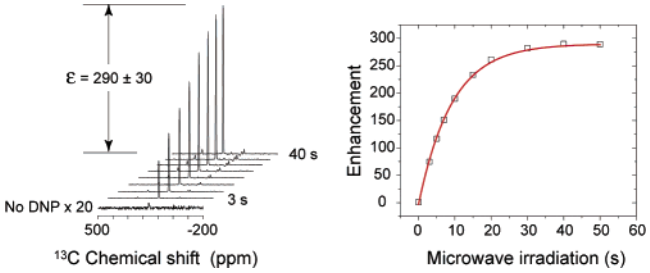

Figure 3. (Left) MAS-DNP experiment illustrating the growth of the polarization in a ^{13}C-urea sample after microwave irradiation. The irradiation period extends to ~40 s and an enhancement of 290 ± 30 is achieved. (Right) Plot showing the development of the polarization enhancement with time. The growth time constant is ~9 s.

in the melting experiment with those obtained from room-temperature solution NMR experiments. The room-temperature liquid-state spectra were directly detected and typically acquired by averaging 128−512 scans with a long recycle delay (60−120 s) to ensure that we reached the equilibrium Boltzmann polarization. Note that in generating the DNP-enhanced ^{13}C signals, we transfer polarization from electrons to ^{1}H and then to ^{13}C via CP since this is the most time-efficient manner to move polarization from the electrons to the ^{13}C. In principle, we could polarize ^{13}C directly, but the process is slower since spin diffusion in the ^{13}C reservoir is slow. It should also be possible to detect the ^{13}C signals indirectly via observation of ^{1}H, as is customary in many solution NMR experiments,[38] and we will describe those approaches in future publications.

3. Results and Discussion

In Figure 3, we illustrate a series of spectra of ^{13}C-urea obtained with a magic angle spinning experiment as a function of the microwave irradiation period. The spectra illustrate the growth of the ^{13}C polarization to a value 290 ± 30 times that of the Boltzmann polarization. The initial trace in the series shows the spectrum without microwaves expanded by a factor of 20. For ^{13}C-urea in water/DMSO, the polarization process reaches equilibrium in ~40 s. In the case of ^{13}C-urea, we achieve $\epsilon = 290 \pm 30$ of the ^{1}H polarization, which implies a larger enhancement of ^{13}C polarization. However, in other samples this value is lower since relaxation processes are present.

Figure 4 shows the TJ-DNP-enhanced ^{13}C NMR spectra of ^{13}C-urea, Na[1,2-$^{13}C_2$,$^{2}H_3$]-acetate, and [U-^{13}C,$^{2}H_7$]-glucose. The top traces of each panel illustrate the TJ-DNP-enhanced spectrum, and the lower traces show the signal intensity obtained with ^{1}H-decoupled Bloch decays for comparison. The enhancements observed in the spectra, which we label as $\epsilon^{\dagger}$, a definition that is discussed below, are included for each compound in the figure and are ~400 for urea, ~290 for sodium acetate, and ~120 for glucose. Note the ^{13}C−^{13}C *J*-coupling that is resolved in the acetate spectrum. This clearly establishes that, when the ^{13}C T_1 is long compared to the melting period and is sufficiently long in the solution phase, it is possible to observe significant signal enhancements in the ^{13}C solution spectra and that the resolution is not degraded by the presence of a paramagnetic polarizing agent.

We noted above that we have labeled the enhancements as $\epsilon^{\dagger}$, rather than ϵ as is common in solid-state MAS experiments.[2−9] Thus, there are two conventions in use to report the size of enhancements that deserve explanation:

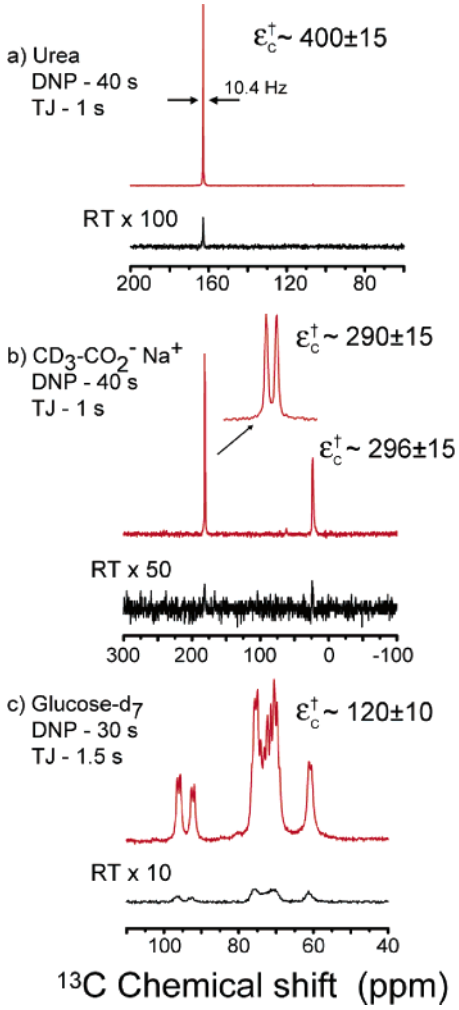

Figure 4. ^{13}C TJ-DNP NMR spectra of (a) ^{13}C-urea in 50% $^{2}H_6$-DMSO and 50% water (80% $^{2}H_2O$/20% H_2O), (b) Na[1,2-$^{13}C_2$,$^{2}H_3$]-acetate in 60% $^{2}H_8$-glycerol and 40% water (80% $^{2}H_2O$/20% H_2O), and (c) [U-$^{13}C_6$,$^{2}H_7$]-glucose in H_2O. Samples contained 3−5 mM TOTAPOL biradical polarizing agent,[21] corresponding to 6−10 mM electrons. As explained in the text, deuteration of the samples was employed in order to circumvent the ^{1}H-mediated ^{13}C relaxation in the viscous solution phase. The times required for polarization and melting of the sample are indicated next to each trace. The TJ-DNP spectra (the top traces in each figure) were recorded with a single scan, while the room-temperature spectra were recorded with (a) 256, (b) 128, and (c) 512 scans, respectively.

(1) In solid-state MAS experiments, it is usual to compare the signal intensity in the presence and absence of microwave irradiation at the temperature where the DNP enhancement is performed. This ratio of signal intensities yields the enhancement ϵ due to the microwave irradiation. The data and enhancements reported in several other publications from this laboratory at $T \leq 90$ K use this convention and are due to the microwave-driven enhancement alone.[1−9]

(2) In the case of liquids, however, the relevant enhancement, that we define as $\epsilon^{\dagger}$, is determined by the intensity of the DNP-enhanced signal relative to the signal due to the Boltzmann polarization recorded at 300 K. Since the polarization is generated at low temperature, for example 90 K, there is an additional factor of $(T_{obs}/T_{\mu wave}) \approx (300\ K/100\ K) = 3$ included in the calculation of the enhancement $\epsilon^{\dagger}$. When the polarization is performed at 1.2 K and the observation is at 300 K, this number increases to 250. Thus, enhancements reported in the literature for solid-state and liquid-state experiments differ by

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the factor ($T_{obs}/T_{\mu wave}$), which can be substantial. For example, by polarizing at 1.2 K, Ardenkjær-Larsen and co-workers[39] reported $\epsilon^{\dagger} = 44\,400$, which corresponds to $\epsilon = 178$ if we take ($T_{obs}/T_{\mu wave}$) = 250. Accordingly, in this paper we quote two enhancements, $\epsilon^{\dagger}$ and ϵ, that are related by

$$\epsilon^{\dagger} = \epsilon(T_{obs}/T_{\mu wave}) \quad (1)$$

where T_{obs} and $T_{\mu wave}$ are the temperatures where the signal observation and microwave irradiation occur. Note that $\epsilon^{\dagger} = \epsilon$ in the limit where $T_{obs} = T_{\mu wave}$.

In Situ Melting and Dissolution Experiments. There are several features of the experiments described here that differ in significant ways from the experiments described by Ardenkjær-Larsen et al.[39] In particular, while we performed an in situ TJ melting experiment, they in contrast utilized an approach involving polarization at low field, "dissolution" of the sample, and transfer to a higher field for observation. The difference in the important experimental details is as follows. First, in the dissolution experiment, the polarization step was performed at 1.2 K rather than 90 K. Second, it was performed in a 3.35 T field using a 200 mW, 94 GHz microwave source to drive the DNP process. Third, the triphenylmethyl-based trityl radical[40] was the polarizing agent, and the ^{13}C spins in the sample were polarized directly ($\epsilon \approx 178$) rather than through the ^{1}H's. Because of the low temperature, the low microwave power, the long T_{1e} of the trityl radical, and the fact that ^{13}C was polarized directly, their polarization times were ~80 min. In contrast, we are able to achieve enhancements $\epsilon \approx 290$ in ~40 s at ~90 K at 5 T using our 140 GHz microwave source and biradical polarizing agents. Finally, in the "dissolution" experiment, the sample, consisting of 40−50 mg of frozen polarized pellets, is melted and dissolved in ~7 mL of hot water, diluting it by a factor of ~150. If the polarized solute is used in imaging experiments, then dilution of the sample with solvent may not be a concern. However, for the analytical experiments that are the focus of this paper, it is clearly undesirable. Following dissolution, the sample was manually shuttled to a 400 MHz liquids spectrometer where the solution NMR spectrum was recorded. Because of the requirement of shuttling to a second magnet, it is not possible to rapidly repolarize the sample. In the results illustrated in Figure 4, the melting and spectroscopy are performed in situ. Further, the sample is not diluted since the melting is performed with a 10.6 μm laser light. Finally, since the polarization and observation are performed in situ, the sample can be refrozen, repolarized, etc. and the experiment recycled in the manner that is customary in analytical NMR experiments. The point is illustrated in Figure 5, where we show a series of 16 spectra acquired over a period of ~40 min from a sample of ^{13}C-proline that was cycled through the steps:

$$[\text{Polarization (40 s)}—\text{Melting (1 s)}—\text{Acquisition (100 ms)}—\text{Refreezing (90 s)}]_n$$

This result illustrates that, even at this early stage in the development of the experiments, the apparatus is sufficiently stable to reproduce the intensities in the spectra to ~5%. We also mentioned above that, in the spectra of the samples that normally contain protons, the ^{1}H's were substituted with ^{2}H. The reason for this is that, in the liquid phase, the glassy glycerol mixtures used to polarize the samples are viscous. Consequently, the ^{1}H relaxation times are very short (milliseconds),[41] and the short ^{1}H T_1 leads to relaxation of the ^{13}C and loss of the ^{13}C signal. However, we can recover the lost signal-to-noise of protonated carbons, such as those in protonated L-proline and sodium acetate, from signal average through recycled TJ-DNP experiments (Figure 5, right). Moreover, preliminary experiments with solvent systems that exhibit lower viscosity in the liquid phase, and still form low-temperature glasses that disperse the biradical, suggest possible circumvention of relaxation problems with employing protonated molecules in the TJ-DNP experiments.

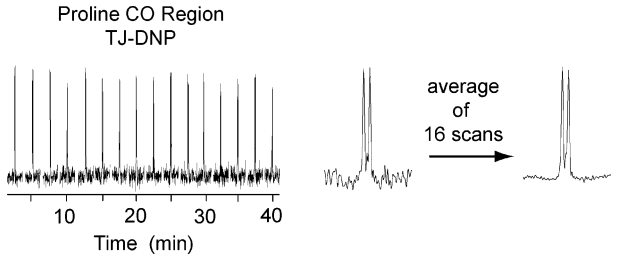

Figure 5. (Left) Sixteen spectra of the carbonyl resonance in [U-^{13}C]-L-proline resulting from a series of TJ-DNP experiments employing the sequence DNP (40 s)—Melting (1 s)—Acquisition (100 ms)—refreezing (90 s). The spectra illustrate that, following melting, the sample can be refrozen and repolarized and another spectrum recorded in order to perform signal averaging. (Right) The 16 spectra can be averaged to show improved signal-to-noise.

Applications of TJ-DNP. The results shown in Figure 4 clearly indicate that, in its present form, TJ-DNP is capable of providing substantial enhancements in sensitivity in ^{13}C and other spectra of small molecules. Thus, when the quantity of sample is small and it can be repeatedly frozen, polarized, and melted, then TJ-DNP experiments could provide a means to acquire ^{13}C spectra with excellent signal-to-noise in relatively short periods of time. An area where the current experimental protocol might find wide application is in metabolic screening, a subject that is of intense interest in the pharmaceutical industry.

Future Refinements. Our purpose here is to demonstrate the feasibility of using TJ-DNP for observing spectra of liquids with enhanced sensitivity. However, at this point the TJ-DNP is at an early stage of development, and there remain many possible technical improvements that could be implemented. Some of the most obvious and potentially significant are to perform the polarization at lower temperatures, to improve the efficiency of the melting process, and to perform the experiments in glassy solvents that have a lower viscosity at room temperature. For example, the spectra displayed in Figure 4 were the result of polarizing at ~90 K, and if biradical electron T_1's are not too long, then it might be possible to polarize rapidly in the 2 K regime and to achieve even larger enhancements rapidly. In addition, once the sensitivity is optimized, the TJ-DNP experiment could be integrated with experiments designed to acquire multidimensional spectra rapidly[42−45] or in a single scan[46] to

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obtain high-sensitivity multidimensional experiments in a few seconds or a fraction of a second.

Finally, we should comment on the applicability of the TJ-DNP approach to solution spectroscopy of proteins and nucleic acids, etc. Clearly, for proteins and nucleic acids, the limiting feature of the present experimental protocol is the freezing and thawing process. If the protein or nucleic acid is robust, then the experiment could be quite useful, and we anticipate that this will be the case for some systems, which are presently under investigation.

4. Conclusions

In summary, we have demonstrated the possibility of observing sensitivity-enhanced ^{13}C spectra of small molecules by first polarizing the sample and then melting it with laser radiation, followed by observation of the solution NMR spectrum. Currently, we utilize biradical polarizing agents and gyrotron microwave sources for the DNP process. The latter enables the experiment to be performed in situ and to be recycled for signal averaging, as is customary in conventional time domain NMR spectroscopy. The sensitivity enhancements at room temperature, where the spectra are observed, are presently ~120−400, but they could be improved by performing the polarization step at lower temperature and by further refining the experiment in the aspects of melting procedures and solvent compositions. In its present form, the experiment is most readily applicable to small molecules and may find applications in metabolic screening.

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