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N,N-Diethylmethylamine as lineshape standard for NMR above 130 K

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ABSTRACT

We demonstrate that N,N-Diethylmethylamine (DEMA) is a useful compound for shimming the magnetic field when doing NMR experiments at room temperature and 130 K, near the temperature used in many dynamic nuclear polarization (DNP) experiments. The resonance assigned to the N-methyl carbon in DEMA at 14.7 T and 140 K has a full-width-half-max linewidth of <4 Hz and has a spin-lattice relaxation time of 0.17 ± 0.03 s.

1. Introduction

Improving the magnetic field homogeneity by adjusting shimming fields has long been a necessary step to ensure narrow peaks and maximum sensitivity in NMR experiments [1]. The samples that are commonly used for shimming NMR magnets at room temperature are not very useful for experiments at temperatures below 150 K, such as in the majority of dynamic nuclear polarization (DNP) experiments, because they typically produce broad (>60 Hz ^{13}C) peaks. It is possible to improve field homogeneity by maximizing the integrated intensity, but unless narrow lines are available, it is hard to find optimal shim parameters. Also, many of the common line shape standards for solid-state NMR such as adamantane have long ^1H and ^{13}C spin-lattice relaxation T_1 (>50 s) at temperatures below 150 K, which would result in a time-consuming shimming protocol. For DNP experiments one common practice is to optimize the shim fields on a standard sample near room temperature and assume that the field remains homogenous at temperatures in the 100–150 K range. However, the ability to shim the magnet while the sample and probe are cold, would allow researchers the agility to adjust for any changes in homogeneity that occur as a result of cooling.

The broad linewidths often observed for low temperature biological samples are an obstacle to the use of low temperature methods such as DNP for many applications [2]. Several studies have shown that inhomogeneous broadening contributes significantly to the peaks widths [3,4], especially for systems with many accessible states; the broadening is often more pronounced for sites at the solvent interfaces [5,6]. Even for more rigid systems, such as the tri-peptide MLF after crystallization, Bajaj and coworkers documented broadened peaks at intermediate temperatures (120–210 K). The broadening was attributed to intermediate time scale motion [7], since most of the peaks are narrower at a lower temperatures. However, even in most well-behaved systems, under standard DNP conditions, peaks are still broadened as compared to room temperature data [7,8]. By contrast, there are a few examples of narrow ^{13}C peaks in DNP enhanced NMR spectra of small molecules at lower temperatures; one notable example is the spectrum of sulfolane at 60 K which has peaks with full-width-half-max (FWHM) on the order of 20 Hz [9].

Given the importance of resolution for modern NMR, it is useful to confirm shim values on a standard sample for studies at low temperature (<150 K). We explore here the conjecture that for at least some instruments, the magnet field homogeneity can be improved by re-shimming after temperature changes. We demonstrate this using N,N-Diethylmethylamine (DEMA) as a lineshape standard at temperatures similar to many DNP experiments. DEMA is a liquid at standard temperature and pressure and it has an unusually low melting point near 130 K [10].

2. Methods

Working in a fume hood, 30 mg of N,N-Diethylmethylamine (DEMA, Aldrich, ≥97%, LOT # BCBK9055V) was pipetted into a Bruker 3.2 mm ZrO_2 rotor, sealed with a Bruker Teflon spacer,

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and capped with a tight-fitting Vespel cap. A small amount of epoxy was applied to the top of the Teflon spacer and on the sides of the cap before closing to ensure that the rotor remained sealed at low-temperature. It is noted that DEMA partially dissolved both Silicon and natural rubber, two commonly used materials for sealing liquids in magic-angle spinning rotors.

NMR experiments were performed using a 600 MHz Bruker Avance III system equipped with a 3.2 mm HCN DNP probe. The magic-angle-spinning rate was 5.0 ± 0.2 kHz for Potassium Bromide and Adamantane and 2.5 ± 0.2 kHz for DEMA measurements. The DEMA sample was spun to lower magnetic susceptibility effects of the rotor [11]. For low-temperature experiments, the temperatures of the variable temperature, drive and bearing N_2 gas were regulated at 140 K. Experiments were run using nitrogen gas at both temperatures. In all carbon detected experiments, 20 kHz Waltz decoupling [12] was applied to remove J_{CH} multiplets. The Waltz decoupling RF parameters were optimized at both temperatures. As expected with DEMA in a liquid phase, cross polarization experiments result in little signal, presumably because of the weak dipolar coupling in the liquid phase; therefore, single pulse experiments were used for DEMA. The probe was cooled for >2 h before running lineshape tests. The magic angle setting was optimized to within 0.1° by maximizing the signal of rotor echoes on Potassium Bromide [13] at both temperatures. The magic angle setting had to be adjusted after cooling the probe, see Fig. S1. Additional pulse sequence parameters are included in the figure captions. Spectra were plotted and analyzed using Python with the nmrglue [14], LMFIT [15] and emcee [16] packages.

3. Results

We searched for a compound with a low melting temperature, in the hope that it would provide narrow linewidths in a liquid phase. After excluding compounds that are gases at room temperature and pressure, because they are difficult to handle and pack into a rotor, we identified DEMA. DEMA is commercially available and relatively easy to handle.

At a set temperature of 295 K, the active shims were optimized using the N-methyl carbon of DEMA. The optimal shim resulted in an N-methyl carbon peak linewidth (FWHM) of 0.6 Hz, see Fig. 1a. Using similar shim settings, the adamantane ^{13}C peak with a higher chemical shift, had a linewidth (FWHM) <1 Hz (see Fig. S2a). By contrast if shims were optimized at room temperature, in spectra of DEMA at 140 K the linewidth (FWHM) of the narrowest peak was 24 Hz, see Fig. S3. This broadening was not corrected by setting the magic angle setting or optimizing decoupling, which resulted in only a moderate reduction of the linewidth to 22 Hz (Fig. 1b). Moreover, the spectrum has a poor line shape with two maxima. The lineshape can be removed by shimming: the N-methyl peak could be narrowed to 4 Hz (FWHM) (Fig. 1c) by adjusting only the Z and X^2-Y^2 magnetic field shims. The N-methyl peak was better fit by a Lorentzian than a Gaussian function (Bayes Factor: >8, [17] see Fig. S4), suggesting that the shimming is close to optimal and that peak broadening mechanisms are homogeneous. The corresponding apparent relaxation time is $T_2^* = 1/(\pi FWHM) = 80$ ms. The ratio between the integral of the N-methyl carbon at 140 K and 295 K was 1.8; close to the ratio predicted by Boltzmann statistics alone of approximately 2.1.

The narrowest DEMA ^{13}C peak (natural abundance) had a signal-to-noise of 118 after 64 scans; this was enough to adjust the active shims efficiently. Under the low-temperature experimental conditions (~140 K), the ^{13}C T_1 measured using inversion recovery was 0.19 ± 0.03 s, 0.17 ± 0.03 s, and 0.24 ± 0.03 s for the methyl, N-methyl, and methylene carbon respectively (±6σ, see Fig. S5). A recycle delay of 3 s was adequate to obtain integrals within 5% of their expected values. For comparison, the FWHM of the narrowest peak in a spectrum of adamantane at 140 K had a FWHM of >60 Hz and had a ^{1}H T_1 of >50 s (see Figs. S2b and S6).

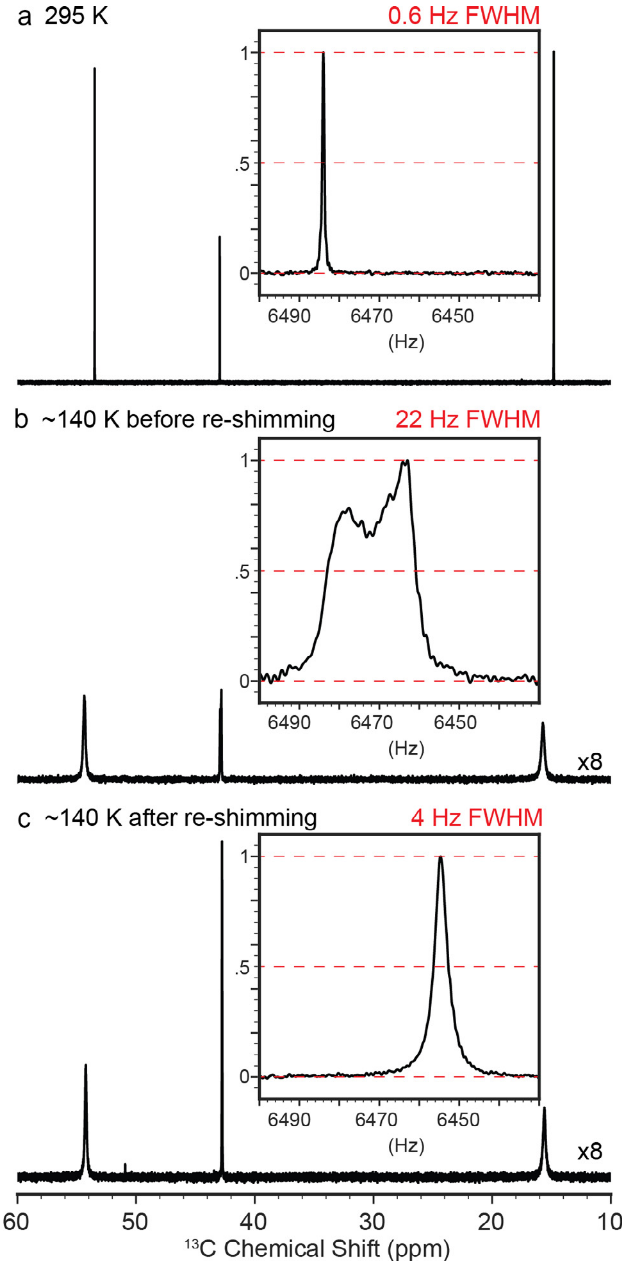

Fig. 1. Direct polarization ^{13}C spectra of DEMA at (a) 295 K, (b) ~140 K before re-shimming, and (C) ~140 K after re-shimming. In the inserts, the chemical shift axis is scaled, and the peak heights are normalized. The peaks were assigned as methyls attached to methylenes (14.8 ppm), N-methyl amine (43.0 ppm) and methylenes (53.4 ppm). The apparent chemical shift change is mostly a result of a change in the field; the spectrum can be re-referenced after shimming by setting the N-methyl peak to 43.0 ppm from DSS, this is the value at room temperature and is equivalent to setting the ^{13}C peak of adamantane with a higher chemical shift to 40.5 ppm. The acquisition times for experiments at 295 K and 140 K were 1.6 s and 1.0 s respectively. The recycle delay was 20s and 3s at 295K and ~140K, respectively.

4. Discussion and conclusion

These results show that DEMA is a useful shimming standard at temperatures near 140 K. Although the linewidth (FWHM of 4 Hz) observed in the DEMA carbon spectra is still broader than that of the best NMR lineshape standards used near room temperature, it is much narrower than typical lines observed for samples of interest in low temperature NMR. One caveat for this method is that low temperature experiments including DNP are often performed at temperatures lower than 140 K, in a range where the spectrum of DEMA is broadened. Until another commonly available compound is found to produce narrower lines at even lower temperatures, DEMA provides a big improvement in available lineshape standards near DNP conditions. DEMA has one of the lowest melting points of known substances that is also a liquid at standard temperature and pressure.

The spectra presented demonstrate that the magnetic field homogeneity should not be assumed to remain constant as a function of temperature. The magnetic field can be altered by possible thermal contraction and temperature-dependent magnetic susceptibility of the probe and rotor materials. The effect of temperature on the optimal shimming conditions will be dependent on the detailed design, and for the case of a Bruker 1.9 mm HCN DNP probe, for example, the effect appears to be much smaller.

Using DEMA as a lineshape standard instead of other standards at low temperature is expected to lower the contribution of applied magnetic field inhomogeneity to the observed lineshapes by a substantial factor, and also lower the uncertainty in the determination of the shimming contribution. Inhomogeneous broadening as a result of poor field homogeneity is not readily distinguishable from many other inhomogeneous broadening mechanisms, including broadening caused by conformational distributions and must be accounted for or ideally removed before interpretation of the origin of peak broadening.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jmr.2017.12.021.

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