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Abstract

Dynamic nuclear polarization (DNP) has become a powerful tool to substantially increase the sensitivity of high-field magic angle spinning (MAS) solid-state NMR experiments. The addition of dissolved hyperpolarizing agents usually results in the presence of solvent signals that can overlap and obscure those of interest from the analyte. Here, two methods are proposed to suppress DNP solvent signals: a Forced Echo Dephasing experiment (FEDex) and TRAnsfer of Populations in DOuble Resonance Echo Dephasing (TRAPDORED) NMR. These methods reintroduce a heteronuclear dipolar interaction that is specific to the solvent, thereby forcing a dephasing of recoupled solvent spins and leaving acquired NMR spectra free of associated resonance overlap with the analyte. The potency of these methods is demonstrated on sample types common to MAS-DNP experiments, namely a frozen solution (of *L*-proline) and a powdered solid (progesterone),

both containing deuterated glycerol as a DNP solvent. The proposed methods are efficient, simple to implement, compatible with other NMR experiments, and extendable past spectral editing for just DNP solvents. The sensitivity gains from MAS-DNP in conjunction with FEDex or TRAPDORED then permits rapid and uninterrupted sample analysis.

1. Introduction

The combination of dynamic nuclear polarization (DNP) with high-resolution solid-state nuclear magnetic resonance (ssNMR)[1] has led to previously-inaccessible insights into biomolecules[2] as well as materials' surfaces.[3] High resolution is obtained through the combination of strong magnetic fields (> 5 T) and magic angle spinning (MAS[4]), and DNP is achieved through suitable microwave (μw) irradiation of unpaired electron spins, which permits the transfer of the intrinsically large spin polarization of these spins to nearby nuclei.[5,6] This polarization increase experienced by the nuclei directly translates into signal(-to-noise) gains in NMR experiments. Therefore, ssNMR experiments that would have required days, months, or even years of signal averaging without DNP can now be performed in a small fraction of the time, making them readily practicable. As such, two-dimensional (2D) correlation experiments eluding to internuclear proximities, and thus atomic-level structures, can now be recorded in short experimental time for nuclear spin-pairs using their low natural isotopic abundance (i.e., without isotopic enrichment), for example ^{13}C–^{13}C,[7,8] ^{13}C–^{15}N,[9] and ^{29}Si–^{29}Si[10] (with the natural abundance of ^{13}C, ^{15}N, and ^{29}Si being 1.1%, 0.4%, and 4.7%, respectively).

Since DNP requires the presence of unpaired electron spins, which are not present in most samples, it is usually necessary to introduce exogenous "polarizing agents" containing these spins

to the analyte. For efficient DNP, and also subsequent ssNMR experiments, these polarizing agents should be homogeneously distributed in an optimal concentration.[11] To achieve a homogeneous distribution, exogenous polarizing agents are normally dissolved in a glass-forming solvent or solvent mixture, which is then added to the analyte. Although glassing-matrices with beneficial properties for MAS-DNP experiments,[12] such as glycerol/water,[1] dimethylsulfoxide/water,[13] 1,1,2,2-tetrachloroethane,[12] 1,1,2,2-tetrabromoethane,[12] and ortho-terphenyl,[14] have all been successfully utilized, they all contain NMR-active nuclei and therefore exhibit resonances that can overlap with signals of interest from the analyte in acquired spectra. ^{13}C ssNMR is particularly important since it is crucial for studies of organic molecules (biomolecules, pharmaceuticals, etc.), but also material science (organic-inorganic hybrids, polymers, molecular organic frameworks, etc.). So the employment of glycerol, dimethylsulfoxide (DMSO), 1,1,2,2-tetrachloroethane (TCE), 1,1,2,2-tetrabromoethane (TBE), and ortho-terphenyl (OTP) can be detrimental as they all contain carbon nuclei. The most ubiquitous mixture is glycerol/water, specifically glycerol-d_8/D_2O/H_2O (60/30/10; v/v/v), since it can be used with the vast majority of systems, acts as a cryoprotectant, and forms a good glass at temperatures < 140 K.[15] Low temperatures (< 200 K) are conventionally used for DNP as they result in increased DNP efficiency[16] due to better glasses and longer spin relaxation times and coherence lifetimes.[11] To reduce the problem of spectral overlap for MAS-DNP studies involving ^{13}C ssNMR, ^{13}C-depleted deuterated glycerol[17,18] has been used. However, this is an expensive work-around ($^{12}C_3$ (99.95 %), D_8 (98 %), ~$650 per gram at the time of writing). Some sample preparation procedures, such as incipient wetness impregnation[3] and the Matrix-Free[7,19] approach, can limit the amount of DNP matrix used, while still retaining DNP efficiency. However, these procedures can still result in significant solvent signals and are not suitable for all possible analytes.

Spectral editing can be used to remove unwanted signals in NMR spectra. Spectral editing involves the use of data processing techniques and/or acquisition methods to retain only the desired signals.[20,21] The use of specific NMR pulse sequences, for example, can allow the favoring of certain signals over others, usually by modifying the intensity or phase of these others. In solution-state NMR, this type of spectral editing is routinely used to remove solvent signals. For the suppression of solvent resonances in solution-state ^{1}H NMR, various pulse sequences have been proposed[21] and these generally involve the selective saturation of signals at a particular finite frequency, which corresponds to that of the solvent signals. However, this method is not suitable for the present problem since solution-state solvent signals are narrow and signals that are overlapping with (i.e., at the same frequency as) these solvent signals are not recovered. The ^{13}C NMR signals arising from solvent molecules in a frozen glass are (heterogeneously) broad (typically ~5-10 ppm) and thus there could be many ^{13}C resonances from the analyte within the same frequency range. Therefore, it is necessary to suppress the solvent signals in a different manner. Spin-echoes have been employed for this purpose,[22] whereby a long MAS-synchronized delay is added either side of a refocusing π-pulse. However, this method is only suitable if the solvent has a much shorter time constant for transverse signal decay under a refocusing echo, T_2', than the analyte.

Since deuterated solvents are commonly used for DNP matrices so that an overall ^{1}H concentration roughly equivalent to the ^{1}H concentration in glycerol-d_8/D_2O/H_2O (60/30/10; v/v/v) is obtained as this gives good DNP efficiency,[15] this then brings a difference between analyte and solvent. It is this difference that can be exploited. For the glycerol/water DNP matrix, the glycerol is conventionally fully deuterated (i.e., glycerol-d_8) and thus all of its carbon nuclei are directly bonded to at least one ^{2}H (see inset in Figure 3). Therefore, spectral editing that involves triple-

resonance ($^{1}H/^{13}C/^{2}H$) experiments could potentially be used in a similar manner to those already used for spectral editing of isotopically-labeled biomolecules through triple-resonance ($^{1}H/^{13}C/^{15}N$) experiments.[23] There, REDOR[24]-type experiments were used to recouple the ^{13}C–^{15}N dipolar interaction, which is decoupled under MAS, so that ^{13}C nuclei in the near proximity (within one bond) of ^{15}N nuclei would be dephased, leaving only ^{13}C signals in the resulting NMR spectrum of sites that are distant from nitrogen-containing moieties. However, REDOR is used to recouple spin-1/2 nuclei, and is thus not appropriate for the work here since ^{2}H is a spin-1 nucleus. For the recoupling of ^{13}C with quadrupolar nuclei (spin > ½), TRAPDOR NMR[25] can be used.

Herein, a spectral editing approach is proposed to remove signals associated with the DNP matrix. Two methods are presented that reintroduce the heteronuclear dipolar interaction to ^{2}H spins during a "dephasing period" so that only resonances from spins that are not coupled to ^{2}H remain in the resulting spectrum. The utility of both methods is presented firstly on a frozen solution of *L*-proline, and then secondly on powdered progesterone. The results demonstrate that the presented technique, combined with MAS-DNP, produces solvent-free spectra with large sensitivity, thus facilitating the fast spectral assignment of analytes.

2. Materials and methods

2.1. Preparation for DNP experiments

A solution of 2 M *L*-proline at natural isotopic abundance, 10 mM AMUPol,[26] and glycerol-d_8/D_2O/H_2O (60/30/10; v/v/v) was prepared and a 20 μL aliquot of this was inserted into a 3.2 mm outer-diameter sapphire sample rotor, which was subsequently sealed with a silicone plug and closed with a zirconia drive cap. The progesterone sample was prepared by gently hand-

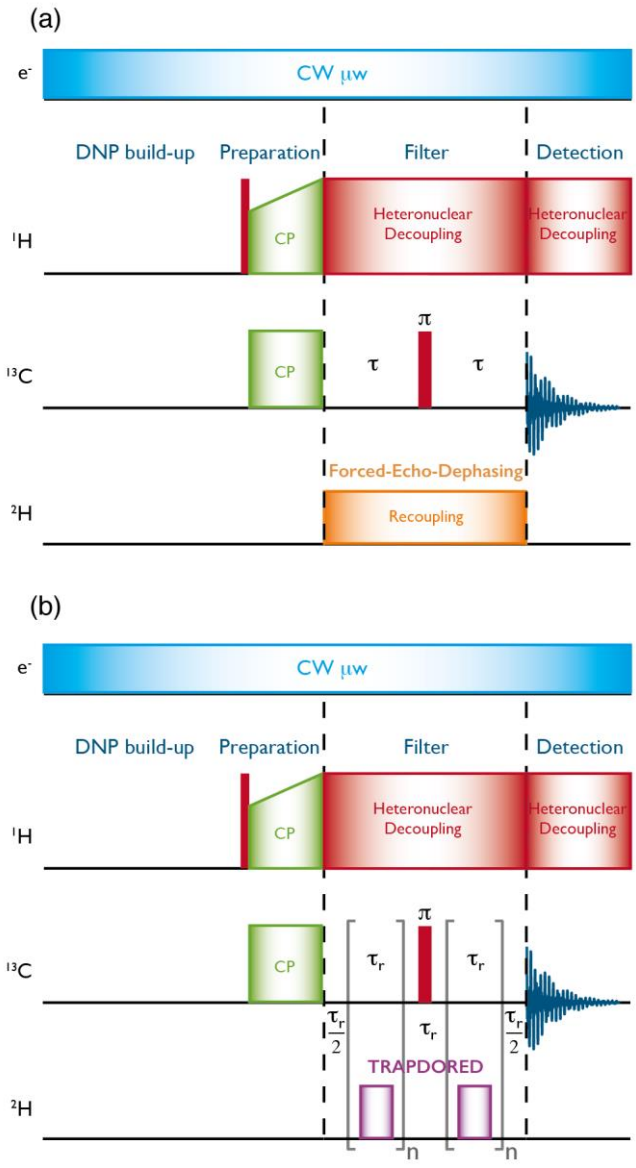
grinding the white solid and slowly adding 10 mM AMUPol and glycerol-d_8/D_2O/H_2O (60/30/10; v/v/v) solution until the powder appeared slightly wet. The damp powder was then fully packed (~30 mg) into a 3.2 mm outer-diameter thin-wall zirconia sample rotor, which was then sealed only with a Vespel drive cap. The sample-containing rotor was inserted into a pre-cooled (~100 K) MAS-DNP triple-resonance ($^1H/^{13}C/^2H$) NMR probe and pneumatically spun to the required rotation rate, ν_r, using cold nitrogen gas. Under these MAS conditions, a few watts of microwave (μw) irradiation at approximately the Larmor frequency of the unpaired electron spins of AMUPol (~ 264 GHz) are applied to the sample, which enables the transfer of the large spin polarization of the unpaired electrons to nearby nuclei. For ^1H, ^1H–^1H dipolar couplings facilitate the equilibration of this hyperpolarization throughout the whole spin system. The *L*-proline, progesterone, glycerol-d_8 (98% in D atoms) and D_2O (> 99% in D atoms) were standard commercial reagents, and the AMUPol biradical polarizing agent was purchased through SATT Sud Est, Marseille, France.

2.2. DNP-enhanced solid-state NMR experiments

Solid-state NMR experiments were recorded using a Bruker DNP-NMR AVANCE III 400 MHz (9.4 T) spectrometer equipped with a gyrotron and associated transmission line capable of delivering ~ 5 W of ~ 264 GHz μw irradiation at the sample.[27] All experiments were recorded with a 3.2 mm HXY triple-resonance MAS probe (in λ/4 ^1H transmission mode) at $\nu_0(^1H)$ = 400.33 MHz, corresponding to the maximum ^1H enhancement field position for AMUPol at $\nu_0(e^-)$ = 263.7 GHz, with the X-channel tuned to $\nu_0(^{13}C)$ = 100.66 MHz and the Y-channel tuned to $\nu_0(^2H)$ = 61.45 MHz.

Figure 1 illustrates the NMR radio frequency (RF) pulse sequences used herein. ^1H π/2 and SPINAL-64[28] heteronuclear decoupling were applied to induce a nutation frequency of 100 kHz.

^{13}C π-pulses and CP spin-locking were at ~ 43 kHz with corresponding ramped (50–100%) ^{1}H spin-locking at ~ 80 kHz (100%) to induce efficient CP transfer. For the recoupling of ^{2}H–^{13}C dipolar interactions, continuous ^{2}H RF irradiation was applied during a rotor-synchronized spin-echo of total duration 2τ (Figure 1a) or as rotor-synchronized pulses of duration ≤ τ_r ($\tau_r = 1/\nu_r$) during a spin-echo with a total duration of $2\tau_r(n+1)$ (Figure 1b). The pulse sequence given in Figure 1a then results in a Forced Echo Dephasing experiment, or FEDex, and the sequence given in Figure 1b a modified version of TRAPDOR,[25] here a TRAnsfer of Populations in DOuble Resonance Echo Dephasing, or TRAPDORED, NMR. Note that in conventional TRAPDOR NMR the RF irradiation applied to the quadrupolar spins is not usually applied in the second half of the spin-echo.[29] For TRAPDORED NMR, optimum dephasing results are obtained with irradiation on the quadrupolar nucleus (here ^{2}H) in both halves of the spin-echo.



145

146 **Figure 1. FEDex (a) and TRAPDORED (b) NMR pulse sequences.** After a DNP build-up
147 period using suitable continuous wave (CW) μw irradiation, transverse ^{13}C magnetization is
148 prepared through a cross-polarization step. Subsequently, a filter period is applied whereby ^{2}H–
149 ^{13}C dipolar interactions are reintroduced to rapidly dephase ^{13}C signals from deuterated solvents,
150 before detection of the remaining ^{13}C magnetization.

151

3. Results and discussion

3.1. Reintroducing the ^{2}H–^{13}C dipolar interaction for forced signal dephasing

Under even moderate MAS rates, the dipolar interaction between ^{2}H and ^{13}C spins will be effectively averaged to zero. Therefore, to produce dephasing of magnetization from ^{13}C nuclei in close proximity to ^{2}H nuclei, the dipolar interaction needs to be reintroduced. Fittingly, a powerful technique that reintroduces the dipolar interaction between spin-1/2 and quadrupolar nuclei under MAS has been previously described. [25,29,30] Figure 1 shows the NMR pulse sequences for the two methods presented herein to dephase deuterated solvent signals and recover only the signals of interest. Both methods rely on employing a filter period where unwanted signals are forcibly dephased due to the reintroduction of a dipolar interaction to ^{2}H spins. The two methods differ in the way in which they reintroduce this dipolar interaction during the filter period. The Forced Echo Dephasing experiment, or FEDex, shown in Figure 1a uses continuous wave (CW) ^{2}H RF irradiation during the filter period, whereas the TRAnsfer of Populations in DOuble Resonance Echo Dephasing, or TRAPDORED, experiment shown in Figure 1b uses rotor-synchronized ^{2}H RF pulses.

Figure 2 presents the experimental optimization of the variables associated with FEDex and TRAPDORED. They are simple to set up, since both only rely on one main variable. For FEDex, this variable is the RF power applied to the ^{2}H spins, which is MAS dependent (see Figure 2a). At the slower spinning rate of 8 kHz, the RF power required for optimum dephasing (i.e., smallest returned peak intensity) is lower than that at 14 kHz, indicating that the dipolar recoupling is due to a matching of the nutation of the ^{2}H spins and the spinning rate (or integer multiple of). Conversely, for TRAPDORED, the maximum possible RF power applied to the ^{2}H spins results in the best dephasing (data not shown), as would be expected with TRAPDOR-type recoupling,[25]

with the optimized variable being the pulse length. It is clear from Figure 2b that the TRAPDORED pulse length should be between $0.5\tau_r$ and τ_r. The dephasing efficiency is fairly robust to the offset frequency of the ^{2}H RF irradiation (± 8 kHz), with best performance slightly off resonance (+ 3 kHz) (see Figure S1 of the Supporting Information). Once FEDex and TRAPDORED have been optimized, then the required dephasing time can be obtained.

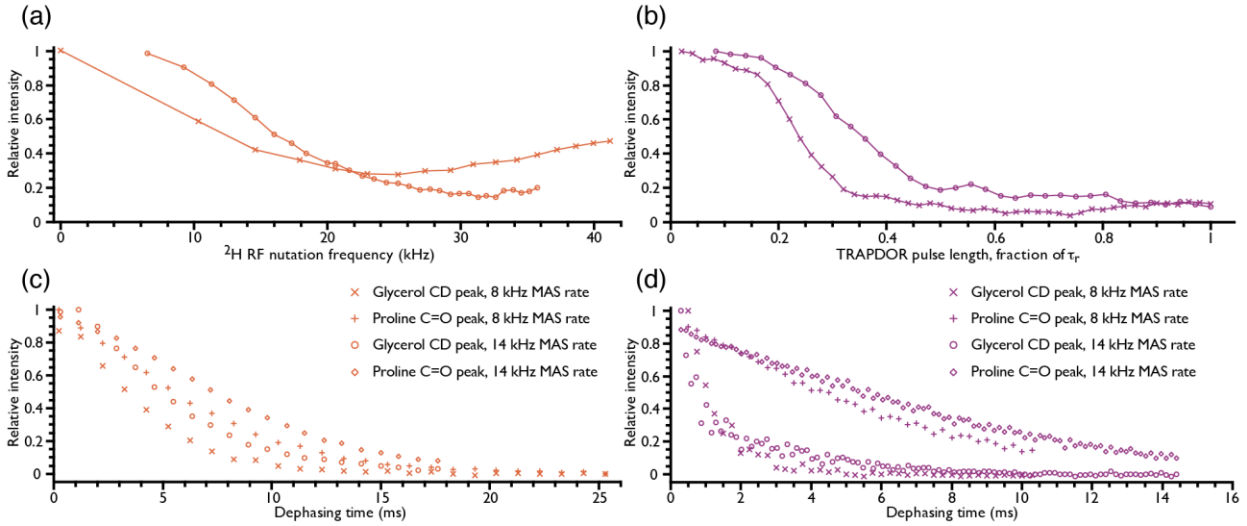


Figure 2. Optimization of FEDex (a, c) and TRAPDORED (b, d) pulse sequences. The sample used was 2M *L*-proline in glycerol-d_8/D_2O/Htop_2O with 10 mM AMUPol. The calibration was performed on the resulting relative ^{13}C intensity of the glycerol CD resonance at $\delta\{^{13}C\}$ = 72 ppm and compared (in (c) and (d)) to the *L*-proline carboxyl ^{13}C resonance ($\delta\{^{13}C\}$ = 176 ppm). A MAS rate of 8 kHz or 14 kHz and recycle delay of 3 s were used, along with total dephasing times of 5.5 ms for (a) or 12.5 ms for (b). A ^{2}H RF nutation frequency of 36 kHz was used for the calibration shown in (b) and (d), whereas 23 and 33 kHz were used for the points measured in (c) at 8 kHz and 14 kHz MAS rates, respectively. Pulse lengths of $0.62\tau_r$ and $0.99\tau_r$ were used for the points measured in (d) at 8 kHz and 14 kHz MAS rates, respectively.

Figure 2c and Figure 2d show the relative intensity of the ^{13}C CD resonance of deuterated glycerol ($\delta\{^{13}C\}$ = 72 ppm, see Figure 3) and compare it to that of the *L*-proline carboxyl ($\delta\{^{13}C\}$ = 176 ppm, see Figure 3) from a frozen solution of 2M *L*-proline, 10 mM AMUPol, and glycerol-d_8/D_2O/H_2O as a function of the filter period duration (dephasing time), recorded using the (optimized) pulse sequences from Figure 1a and Figure 1b, respectively. Instantly, it is evident that TRAPDORED gives better results. For the two MAS rates tested (8 and 14 kHz), there is a greater intensity difference between the unwanted (CD) and wanted (carboxyl) peaks using TRAPDORED. This means that the dephasing is more efficient with this technique. At 8 kHz MAS rate, 5.5 ms of TRAPDORED are required for there to be negligible remaining intensity of the solvent peak, whereas over twice this dephasing time is needed for the same result with FEDex. Therefore, the desired (*L*-proline) signal will be more intense following TRAPDORED, while the solvent signal is suppressed adequately. Note that the proportion of the remaining, desired signal depends on the apparent time constant for transverse signal decay under a refocusing echo, T_2', for the particular resonance. In the case of the *L*-proline carboxyl, 42 % of the signal remains after an echo of 5.5 ms total duration compared to that obtained with a similar experiment recorded without the filter period. It should be highlighted though that the $^{13C}T_2$' for small molecules dissolved in frozen glycerol/water solutions that contain paramagnetic polarizing agents suitable for MAS-DNP is generally short (and depends on the concentration of the polarizing agent).[11] Moreover, there will be a non-negligible recoupling of the ^{2}H–^{13}C dipolar interaction between the abundant glycerol deuterons and the ^{13}C of *L*-proline owing to the dilution of the analyte, which will also induce a shorter apparent $^{13C}T_2$'. Therefore, and as shown below, bulk systems such as crystalline solids that are not as detrimentally impacted by the presence of the paramagnets or recoupling can

retain long $^{13C}T_2$' values, resulting in the acquisition of desired signals with almost negligible intensity losses, while completely suppressing solvent signals, via FEDex or TRAPDORED NMR.

It can also be seen from Figure 2c and Figure 2d that faster sample spinning has a detrimental effect on the efficacy of both dephasing methods, with more relative intensity of the glycerol peak remaining after equivalent dephasing times. Thus, longer dephasing times are required to completely suppress deuterated solvent signals, which could lead to reduced remaining intensity of the desired signals. However, it is also known that faster sample spinning can also increase T_2' values in MAS-DNP experiments,[31] so the losses from desired signals will be less significant. For the example from Figure 2d, 8.8 ms of TRAPDORED at a MAS rate of 14 kHz are required for there to be negligible remaining intensity of the solvent peak, leaving 34 % of the intensity for the desired peak compared to a similar experiment recorded without the filter period. Faster sample spinning reduces the probability of transitions between the energy levels in the quadrupolar ^{2}H nuclei under irradiation, reducing the TRAPDOR recoupling effect.[25] This is due to the irradiation not fully inducing adiabatic population transfers between the Zeeman levels of the ^{2}H nuclei.[25] Therefore, FEDex could be the better choice at higher spinning rates (> 20 kHz).

3.2. Frozen solution of *L*-proline at natural isotopic abundance

Taking the optimized variables and dephasing times from Figure 2 that successfully suppress the ^{13}C signals from the deuterated solvent, DNP-enhanced FEDex and TRAPDORED NMR spectra of *L*-proline in glycerol-d_8/D_2O/H_2O were recorded. *L*-proline was chosen because it has an overlapping ^{13}C resonance with one of the two glycerol ^{13}C peaks (at $\delta\{^{13}C\}$ = 62 ppm). Figure 3 shows these FEDex and TRAPDORED spectra and compares them to corresponding CP spectra. It is clear that both FEDex and TRAPDORED effectively suppress the solvent (glycerol)

^{13}C signals, albeit with intensity losses for the analyte (*L*-proline) – moreso with FEDex than TRAPDORED. These methods then reveal the *L*-proline ^{13}C resonance at $\delta\{^{13}C\}$ = 62 ppm, which was previously obscured by a glycerol ^{13}C peak. As shown in Figure 3, a $\{^{1}H–\}^{13}C$ CP MAS experiment that uses a short CP contact time (50 μs) can also be used to suppress the glycerol ^{13}C signals, since the corresponding nuclei are far from ^{1}H nuclei. However, it does not do so completely, and a short CP contact time will also dramatically reduce the intensity of desired ^{13}C peaks in the analyte that are not directly bonded to ^{1}H, as can be seen from the almost negligible intensity of the carboxyl peak of *L*-proline in Figure 3 (at $\delta\{^{13}C\}$ = 176 ppm).

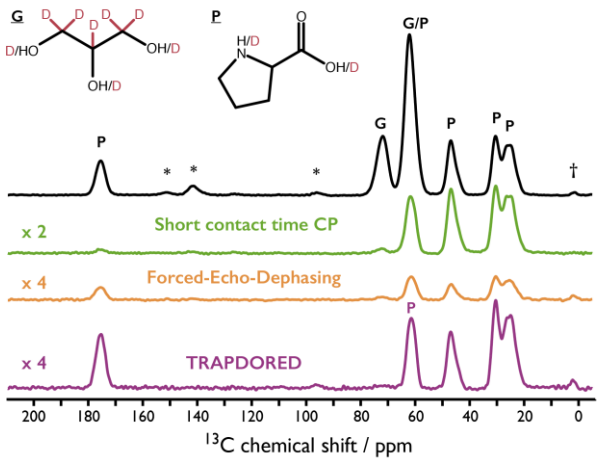


Figure 3. DNP-enhanced ^{13}C NMR spectra of *L*-proline (P, inset) in glycerol-d_8(G, inset)/D_2O/H_2O with 10 mM AMUPol biradical polarizing agent, recorded using a recycle delay of 3 s, a MAS rate of 8 kHz, a sample temperature of ~100 K, and at a magnetic field strength of 9.4 T. A $\{^{1}H–\}^{13}C$ CP MAS NMR spectrum recorded with a CP contact time of 2 ms (black) is compared to a similar spectrum recorded with a CP contact time of 50 μs (green) and FEDex (orange) and TRAPDORED (purple) spectra, both recorded using a CP step with contact time 2 ms. The FEDex and TRAPDORED times were 12.5 and 5.5 ms, respectively. Asterisks (*) and

252 daggers (†) denote spinning side bands and signals from a silicone plug, respectively. Note the
253 factors highlighting the change in scale of the spectra.

254

255 The filter periods from Figure 1a and Figure 1b, representing the FEDex and TRAPDORED parts
256 of the NMR pulse sequence, respectively, can be combined with any solid-state NMR experiment
257 (so long as there are a sufficient number of available RF channels), allowing the suppression of
258 resonances from molecules containing quadrupolar spins. Figure 4 presents DNP-enhanced two-
259 dimensional ^{1}H–^{13}C dipolar correlation spectra of the *L*-proline sample, which have been recorded
260 with (Figure 4b) and without (Figure 4a) the inclusion of ^{2}H TRAPDORED into the pulse
261 sequence. Instantly, it is evident in Figure 4a that there are large cross-peaks stemming from the
262 glycerol (labelled 'G' in the figure), whereas these are not present in Figure 4b, owing to the use
263 of TRAPDORED. Not only do these glycerol resonances obscure information in the ^{13}C
264 dimension, but also in the ^{1}H dimension. Although the ^{13}C nuclei of the deuterated glycerol are
265 not directly bonded to ^{1}H nuclei, they still experience a dipolar interaction with those that are
266 sufficiently close (e.g., from H_2O in the solvent mixture), resulting in the dominating cross-peaks,
267 even at the relatively short mixing time used (250 μs). The ^{1}H dimension of DNP-enhanced ^{1}H–
268 ^{13}C dipolar correlation spectra has proven especially useful in the atomic-scale characterization of
269 functionalized surfaces,[3] so it is extremely important that this remains as free from overlapping
270 solvent signals as the ^{13}C dimension.

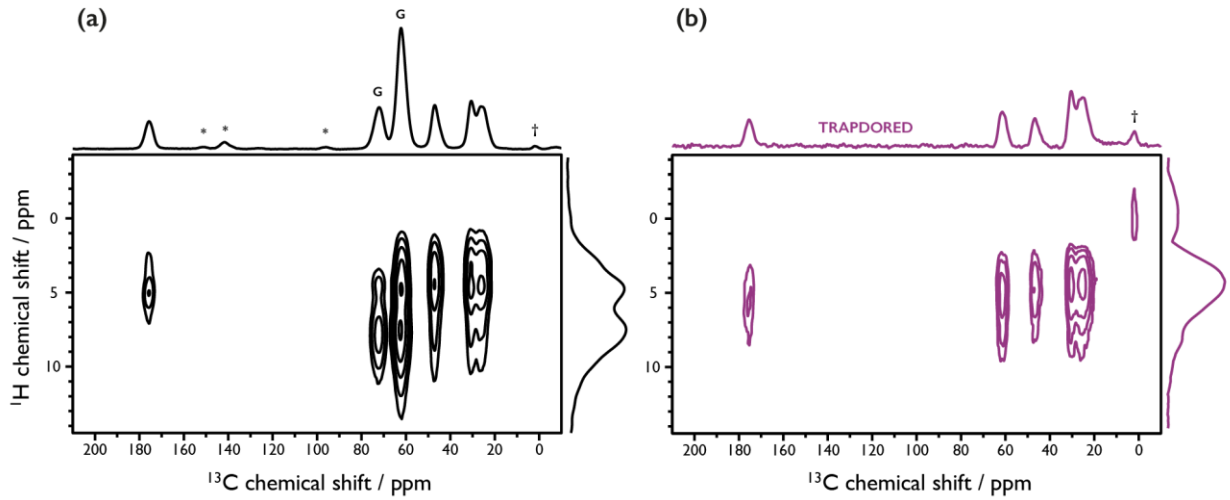


Figure 4. DNP-enhanced ^{1}H–^{13}C FSLG-HETCOR[32] spectra of *L*-proline in glycerol-d_8/D_2O/H_2O with 10 mM AMUPol biradical polarizing agent, recorded using a recycle delay of 3 s, a MAS rate of 8 kHz, a sample temperature of ~100 K, and at a magnetic field strength of 9.4 T. A standard ^{1}H–^{13}C FSLG-HETCOR spectrum (a) is compared to a TRAPDORED version (b), both recorded with CP contact times of 500 μs. Also shown, skyline projections taken parallel to the ^{1}H dimension (right) and corresponding ^{13}C spectra from Figure 3 (top). Asterisks (*) and daggers (†) denote spinning side bands and signals from a silicone plug, respectively.

3.3. Powdered progesterone at natural isotopic abundance

Above, FEDex and TRAPDORED were validated on a *L*-proline frozen solution. There, the intensity losses of the desired signals will be maximized due to their short T_2' values induced by the close proximity of paramagnets to the analyte. Therefore, it is interesting to also demonstrate the usefulness of these methods on a characteristic 'real' analyte, such as a bulk solid that may then not be as negatively impacted by the presence of paramagnets. MAS-DNP has recently be

shown to be immensely valuable for the study of this type of system.[7,8,33,34] Of particular note is the application towards pharmaceutical compounds.[35] Along these lines, Figure 5 shows a DNP-enhanced $\{^1H–\}^{13}C$ CPMAS NMR spectrum of microcrystalline progesterone. The sample preparation, requiring the addition of a glycerol-d_8/D_2O/H_2O solvent matrix to allow dispersal of the polarizing agent and efficient hyperpolarization of the progesterone, results in large glycerol peaks in the $\{^1H–\}^{13}C$ CPMAS NMR spectrum that overlap with signals from the progesterone. Also shown in Figure 5 are corresponding spectra that have been recorded with the pulse sequences in Figure 1a and Figure 1b, giving FEDex and TRAPDORED spectra, respectively. The solvent peaks have been effectively suppressed in these spectra. Moreover, there are only small or negligible signal losses from the analyte. This is because the $^{13C}T_2$' of the progesterone is relatively long, since most of this analyte's nuclei are far from the exogenous paramagnets or ^2H nuclei.

The absolute sensitivity ratio (ASR)[7] for the DNP-enhanced experiment was measured in the usual manner by comparing the optimum signal-to-noise (S/N) ratio per unit square root of time for the DNP-enhanced $\{^1H–\}^{13}C$ CPMAS NMR spectrum to the corresponding spectrum recorded under conventional means (9.4 T, ambient temperature, fully packed 3.2 mm thin-walled zirconia rotor with pure (no solvent or polarizing agent) progesterone powder; data not shown). Here, the ASR = 6. Because the ASR uses a comparison to a conventional experiment, then both the positive and negative effects from performing MAS-DNP experiments are accounted for.[36] An ASR = 6 corresponds to being able to record experiments 36 times faster. Note that previously, using conventional solid-state NMR, 10 days of experimental time was required to record a ^{13}C homonuclear correlation spectrum of progesterone at its natural isotopic abundance.[37] The combination of MAS-DNP and spectral editing, in the form of FEDex or TRAPDORED, therefore allows the fast and undisturbed characterization of analytes.

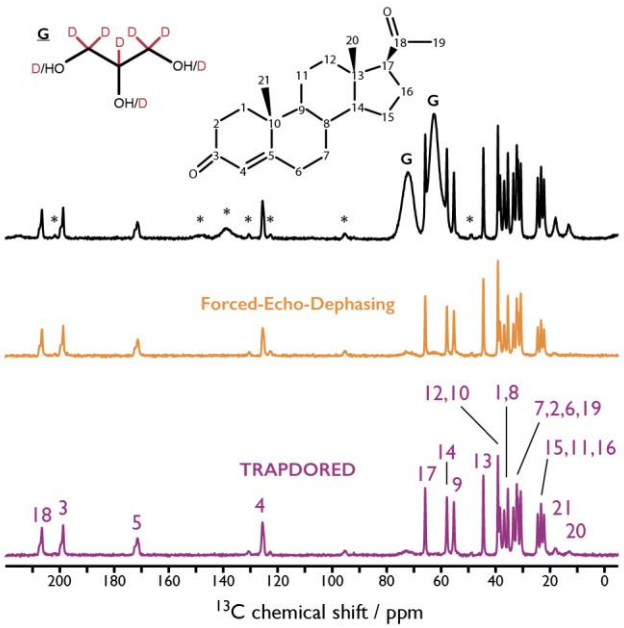


Figure 5. DNP-enhanced {^{1}H–}^{13}C CP MAS NMR spectra of progesterone, wetted with 10 mM AMUPol biradical polarizing agent in glycerol-d_8/D_2O/H_2O, recorded using a recycle delay of 8.5 s, a MAS rate of 7.7 kHz, a sample temperature of ~100 K, and at a magnetic field strength of 9.4 T. A {^{1}H–}^{13}C CP MAS NMR spectrum (black, top) is compared to FEDex (orange, middle) and TRAPDORED (purple, bottom) spectra, all recorded using a CP step with contact time 2 ms. The FEDex and TRAPDORED times were 12.9 and 3.6 ms, respectively. Asterisks (*) denote spinning side bands.

4. Conclusions

Two methods have been presented that force the suppression of solvent signals in common MAS-DNP experiments. These two methods, named FEDex and TRAPDORED, are simple to set up and can be easily combined with established solid-state NMR experiments. They both work through the reintroduction of the heteronuclear dipolar interaction between ^{13}C spins from the DNP

solvent, the presence of which can cause overwhelming signals from associated resonances in ^{13}C NMR spectra, and nearby quadrupolar spins, resulting in fast dephasing of these ^{13}C resonances. The utility of these two methods has been demonstrated for samples containing deuterated glycerol as part of the DNP solvent mixture and for analytes in frozen solution as well as in powdered form. It was shown that TRAPDORED delivers better efficiency than FEDex for solvent suppression under the experimental conditions used here.

It should be highlighted that these methods are not restricted to suppressing signals from glycerol-d_8 and are suitable for other deuterated DNP solvents, many of which have shown useful properties for MAS-DNP studies (such as dimethylsulfoxide-d_6/water,[38] 1,1,2,2-tetrachloroethane-d_2,[39] and ortho-terphenyl-d_{14}[40]). Moreover, the methods proposed herein could also be applied to prevalent non-deuterated MAS-DNP solvents such as 1,1,2,2-tetrachloroethane[3] and 1,1,2,2-tetrabromoethane,[38] where ^{35}Cl or ^{81}Br irradiation could be used instead of ^{2}H irradiation to suppress signals stemming from these solvents, respectively. Finally, the methods demonstrated herein for solvent suppression can also be useful for spectral editing and resonance assignment in conventional solid-state MAS NMR spectroscopy of other systems that contain quadrupolar nuclei in high isotopic abundance, such as ^{7}Li, ^{14}N, and ^{27}Al.

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For Table of Contents Only