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Rapid Acquisition of ^{14}N Solid-State NMR Spectra with Broadband Cross Polarization

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Abstract: Nitrogen is an element of utmost importance in chemistry, biology and materials science. Of its two NMR-active isotopes, ^{14}N and ^{15}N, solid-state NMR (SSNMR) experiments are rarely conducted upon the former, due to its low gyromagnetic ratio (γ) and broad powder patterns arising from first-order quadrupolar interactions. In this work, we propose a methodology for the rapid acquisition of high quality ^{14}N SSNMR spectra that is easy to implement, and can be used for a variety of nitrogen-containing systems. We demonstrate that it is possible to dramatically enhance ^{14}N NMR signals in spectra of stationary, polycrystalline samples (i.e., amino acids and active pharmaceutical ingredients) by means of broadband cross polarization (CP) from abundant nuclei (e.g., ^{1}H). The **BR**oadband **A**diabatic **IN**version **C**ross-**P**olarization (**BRAIN–CP**) pulse sequence is combined with other elements for efficient acquisition of ultra-wideline SSNMR spectra, including **W**ideband **U**niform-**R**ate **S**mooth-**T**runcation (**WURST**) pulses for broadband refocusing, **C**arr–**P**urcell **M**eiboom–**G**ill (**CPMG**) echo trains for T_2-driven S/N enhancement, and frequency-stepped acquisitions. The feasibility of utilizing the BRAIN–CP/WURST–CPMG sequence is tested for ^{14}N, with special consideration given to (i) spin-locking integer spin nuclei and maintaining adiabatic polarization transfer, and (ii) the effects of broadband polarization transfer on the overlapping satellite transition patterns. The BRAIN–CP experiments are shown to provide increases in signal-to-noise ranging from four to ten times and reductions of experimental times from one to two orders of magnitude compared to analogous experiments where ^{14}N nuclei are directly excited. Furthermore, patterns acquired with this method are generally more uniform than those acquired with direct excitation methods. We also discuss the proposed method and its potential for probing a variety of chemically distinct nitrogen environments.

Keywords: ^{14}N NMR · amino acids · broadband cross polarization · pharmaceuticals · solid-state NMR spectroscopy

Introduction

Nitrogen is of great importance in all areas of chemistry and biochemistry. Solid-state NMR (SSNMR) has been used to probe nitrogen sites for over fifty years, with the overwhelming majority of experiments being conducted upon the spin-$^1/_2$ ^{15}N nucleus. The low natural abundance and low gyromagnetic ratio of ^{15}N require, in almost every case, that samples be isotopically enriched to permit the acquisition of high quality spectra within reasonable time frames. There are very few ^{14}N (spin-1) SSNMR studies by comparison, owing to its even lower gyromagnetic ratio and nuclear quadrupole moment ($eQ = 20.44 \times 10^{-21}$ m^2). The quadrupole moment of ^{14}N is particularly troublesome for SSNMR experimentation, as the first-order quadrupolar interaction causes extreme broadening of ^{14}N SSNMR powder patterns in cases where an aspherical ground-state electronic environment causes a sizeable electric field gradient (EFG) at the ^{14}N nucleus.

A number of methods have been explored for the acquisition of ^{14}N SSNMR spectra, including direct observation of ^{14}N NMR signals from single crystals[1] and anisotropically oriented samples,[2] overtone ^{14}N NMR,[3] ^{14}N magic-angle spinning (MAS) NMR with probes with precisely tuned rotor angles,[4] slow spinning and specialized pulse sequences[5] or indirect detection.[6] However, due to requirements of such experiments on the nature of the sample, type of nitrogen environment or technical difficulties with hardware or pulse sequences, none have become widely adopted. There has also been much effort put into the indirect observation of ^{14}N signals by means of spin-coupled spin-$^1/_2$ nuclei (e.g., ^{13}C).[6] For further details on direct excitation and detection of ^{14}N SSNMR spectra, we refer the reader to a recent thorough review on the subject.[7]

We have proposed a methodology for the acquisition of ultra-wideline (UW) SSNMR spectra that has been successful in examining both spin-$^1/_2$ and quadrupolar nuclei.[8] This methodology comprises three components: (i) stepping the transmitter in even increments across the breadth of the

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powder pattern and acquiring sub-spectra at each point (i.e., the variable offset cumulative spectrum (VOCS) or "piece-wise" method, as outlined by the research groups of Massiot[9] and Frydman[10]), (ii) with Carr–Purcell Meiboom–Gill (CPMG) echo trains for enhancing the S/N[11] (i.e., as described for half-integer spin quadrupolar nuclei by Larsen et al.),[12] and (iii) utilizing WURST (Wideband, Uniform Rate, and Smooth Truncation) pulses[13] for uniform excitation of broad powder patterns.[14] The so-called WURST–CPMG pulse sequence has been demonstrated to work for a variety of spin-$^1/_2$[15] and quadrupolar nuclei,[8] and is effective for the rapid acquisition of ^{14}N UW SSNMR spectra for a variety of nitrogen-containing structural moieties.[16] The quadrupolar parameters extracted from the UW SSNMR spectra are extremely sensitive reporters of local nitrogen environments by measurement of the ^{14}N electric field gradient (EFG) tensor.[17] The quadrupolar coupling constant, C_Q, is associated with the spherical symmetry of the ground-state electron density about the quadrupolar nucleus (i.e., as the spherical symmetry is reduced, the absolute magnitude of C_Q becomes larger). The asymmetry parameter, η_Q, describes the axial symmetry of the EFG tensor, and often, the degree of axial symmetry of the local bonding environment. For example, RNH_3^+ groups that are involved in hydrogen bonding typically have moderate values of η_Q (e.g., ca. 0.35–0.65), whereas those that are not have values of η_Q near zero (i.e., ca. 0.0–0.2, axially or near-axially symmetric EFG tensors, with $V_{11} = V_{22}$ or $V_{11} \approx V_{22}$, respectively). It has also recently been demonstrated that ^{14}N powder patterns can be sensitive to molecular motion.[16c] Whereas the information content of ^{14}N SSNMR spectra is high, there are certain types of nitrogen sites that are not amenable to WURST–CPMG ^{14}N direct excitation (DE) experiments, most often due to lengthy longitudinal (T_1) or very short transverse (T_2) ^{14}N relaxation times, low nitrogen weight percentage or combinations of these factors.

One possible solution for dealing with the aforementioned long T_1 relaxation times and natural low-γ limitations is the utilization of cross polarization (CP),[18] which would (i) enhance the ^{14}N NMR signal by transfer of the larger polarization of abundant, high-γ nuclei such as protons and (ii) make the experiment reliant on the ^{1}H T_1s, which are often much shorter than the ^{14}N T_1s. ^{1}H–^{14}N CP experiments have been conducted almost exclusively on single crystals and oriented samples (both standard and overtone experiments),[3a,19] with very few examples of applications to polycrystalline samples.[20] However, a major limitation in conducting CP experiments on UW SSNMR patterns is their narrow excitation bandwidths, which are restricted by the effective bandwidth of the ^{14}N spin-locking pulse and tolerances to offsets from the Hartmann–Hahn matching condition. Even when applying very high power levels (e.g., 300 W or more) to a probe with a 5 mm coil, we have found that typical excitation bandwidths on the ^{14}N channel range from 20 kHz to 50 kHz. The number of sub-spectra that must be acquired to map out ^{14}N powder patterns that are several hundred kHz to a couple of MHz broad is therefore so large that the CP experiment is inefficient in comparison to the broadband DE WURST–CPMG experiment. Obviously, NMR probes with smaller coil diameters would enable somewhat larger CP excitation bandwidths through higher spin-lock power levels, but the reduced sample volume and difficulties in satisfying the Hartmann–Hahn match over a large bandwidth prevents this approach from reaching the efficiency of the WURST–CPMG method. Furthermore, the high power levels utilized in conventional CP experiments must often be applied during the very long contact times expected for a low-γ nucleus like ^{14}N, which puts the probe at increased risk for arcing and potential damage.

Recently, we have demonstrated a method for applying frequency-swept adiabatic inversion pulses for broadband CP to spin-$^1/_2$ nuclei with anisotropically broadened powder patterns.[21] Dubbed the BRAIN–CP (BRoadband Adiabatic INversion Cross Polarization) pulse sequence, it can be used with WURST–CPMG echo trains to produce broadband excitation and yield UW SSNMR spectra with very high S/N. A key advantage of this sequence, aside from its broadband excitation capability, is the ability to perform efficient CP at low radio frequency (rf) power levels, making this pulse sequence especially attractive for use in NMR experiments on low-γ nuclei such as ^{14}N.

Herein, we demonstrate a method using the BRAIN–CP/WURST–CPMG pulse sequence (Scheme 1) for the acquisition of high-quality ^{14}N UW SSNMR spectra, and show results for four samples featuring different types of nitrogen structural motifs (RNH_3^+, aromatic R_3N^+, $RN_2H_2^+$ and R_3NH^+, Scheme 2), including one amino acid and three active pharmaceutical ingredients (APIs). The viability and practicality of the BRAIN–CP method is tested, and shown to yield spectra with S/N enhancements of four to ten times compared to those obtained by means of analogous DE ^{14}N WURST–CPMG experiments with identical CPMG parameters. Spectra acquired with DE and CP methods are compared for each sample, and show an exquisite sensitivity to the different nitrogen environments. Finally, we discuss the potential of this robust and widely-applicable CP experiment in routine use for nitrogen SSNMR on organic, biological and inorganic samples through observation of 99.6% abundant ^{14}N nuclei, without the need for costly ^{15}N isotopic labeling.

Experimental Section

Chemicals. Samples (see Scheme 2) of α-glycine, trigonelline HCl and buflomedil HCl were purchased from Sigma Aldrich and used without further purification. Isoxsuprine HCl was purchased from Sigma Aldrich and recrystallized from D_2O. Samples were powdered and packed into shortened glass NMR tubes (outer diameter, o.d. = 5 mm).

NMR Spectroscopy. ^{14}N SSNMR experiments were carried out on a Varian Infinity+ 400 MHz (9.4 T) spectrometer with $\nu_0(^{14}N) = 28.9$ MHz, equipped with a Varian/Chemagnetics 5.0 mm double-resonance non-spinning HX probe and a low-gamma tuning accessory. A sample of NH_4Cl was used to calibrate the rf power on the ^{14}N channel, as well as to reference the ^{14}N chemical shifts ($\delta_{iso} = 0$ ppm). However,

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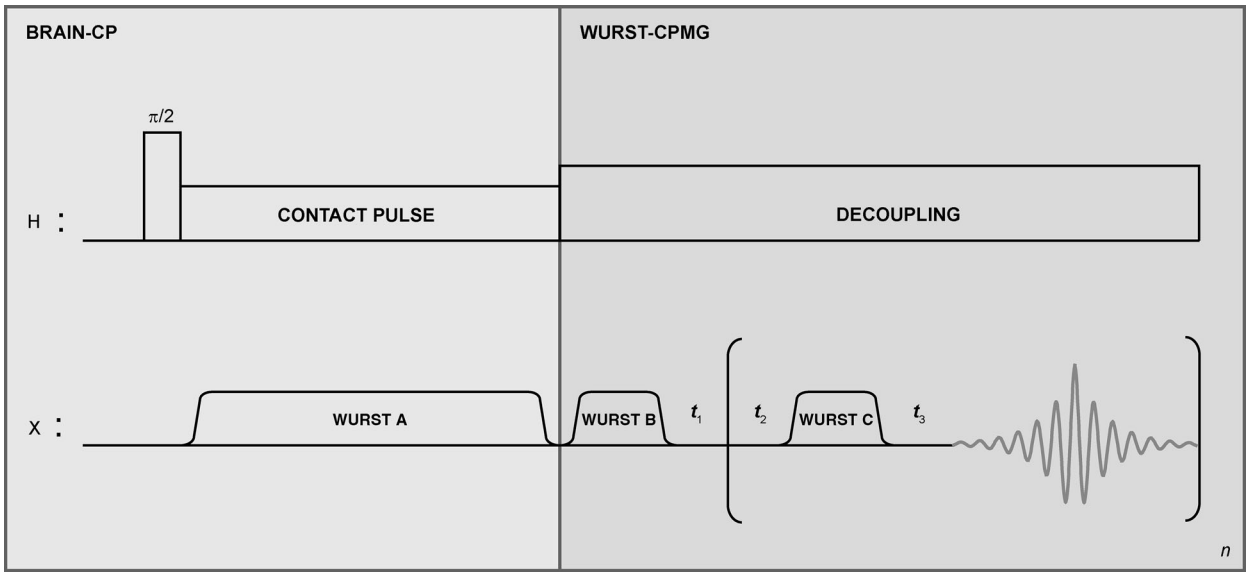
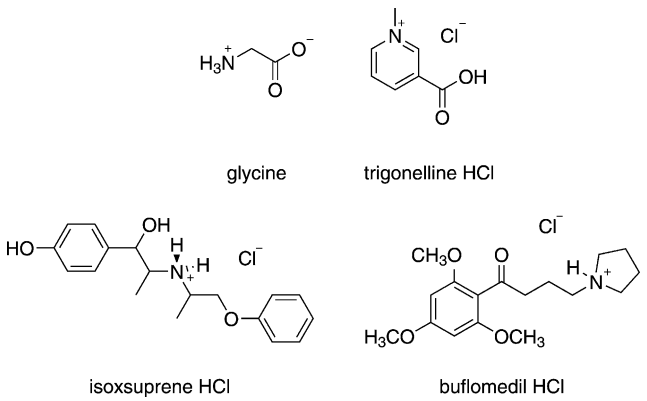


Scheme 1. The BRAIN–CP/WURST–CPMG pulse sequence. The left-hand portion of the sequence is BRAIN–CP, which features a frequency-swept WURST A pulse that fulfills both polarization transfer conditions and an adiabatic storage of the resulting polarization along the z direction, for a wide range of offsets. The right-hand portion of the sequence is WURST–CPMG, which uses the WURST B pulse for rotation of the polarization into the transverse plane whereas a repeated loop of WURST C pulses is used for continued refocusing of the spin polarization.



Scheme 2. The four nitrogen-containing compounds studied in this work.

due to the enormous breadths of the ^{14}N patterns, chemical shifts are not reported, as they have very large uncertainties. This is because the breadths of the quadrupolar patterns discussed herein range from 800 kHz to well over 2 MHz; by comparison, the range of known nitrogen chemical shifts for organic compounds spans about 1300 ppm (ca. 3600 Hz with $\nu_0 = 28.12$ MHz at 9.4 T). Thus, the entire isotropic nitrogen chemical shift range represents less than 0.5% of the breadth of the narrowest ^{14}N patterns discussed herein.

For experiments involving direct excitation of the ^{14}N nuclei, the WURST–CPMG pulse sequence[8] was applied with eight-step phase cycling and frequency-swept WURST 80 pulses[13] of equal amplitude and length for excitation and refocusing. ^{1}H–^{14}N CP NMR experiments were conducted with the BRAIN–CP/WURST–CPMG pulse sequence (Scheme 1). The WURST A pulse is the contact/inversion pulse, which is applied on the ^{14}N channel simultaneously with a conventional rectangular spin-lock pulse on the ^{1}H channel. The WURST B and WURST C pulses are utilized in the CPMG portion of the sequence for excitation and refocusing, respectively. 10 to 12.5 ms WURST 80 pulses with an RF field of 22 kHz were swept over 350 to 500 kHz in a linear fashion for implementing the CP portion of the experiment, and 50 μs WURST 80 pulses swept over 400 kHz were used for conversion and refocusing. In all cases, high-power ^{1}H decoupling was applied, with typical decoupling fields of approximately 40 kHz. All of the spectra presented herein are the best results (highest S/N) after optimizations of CPMG parameters, Hartmann–Hahn matching conditions and contact times. We note that in the event of very short effective T_2 values, BRAIN-CP may be combined with a simple WURST echo experiment to obtain a single spin-echo FID. A two-step phase cycle of the ^{1}H spin lock pulse was used to alternate the generated polarization of the X nucleus between the positive and negative z axes, in order to ensure that only X polarization resulting from CP transfer is observed.[21] We note that after extensive testing of BRAIN–CP with multiple samples and sets of parameters, that glycine is an excellent set-up sample for those interested in initiating trials of this pulse sequence for use with ^{14}N. We provide parameters in the supporting information (see Tables S1 and S2) that should enable easy set-up of glycine (and a number of other samples) on NMR spectrometers of any field strength. The only parameters that require careful optimization are the rf fields for the Hartmann–Hahn matching condition and the sweep widths for the contact and refocusing pulses (fortunately, the acquisition of high quality spectra is quite insensitive to missets in the latter parameters).

Due to the large breadths of the ^{14}N powder patterns and the limited excitation bandwidths associated with the WURST 80 pulses, all spectra herein were acquired with the variable-offset cumulative spectrum (VOCS) method.[9] The transmitter frequency was stepped in even increments across the pattern, with frequency increments equal to an integer multiple of the spikelet spacings arising from the CPMG portion of the pulse sequence.[22] Individual free-induction decays (FIDs) were transformed to produce sub-spectra, which were skyline projected or co-added to produce the total spectra. For all cases but α-glycine, only one half of the overall Pake doublet was acquired in both the DE and CP experiments. Under the assumption of a dominant first-order quadrupolar interaction, the total Pake doublet has mirror symmetry, and can be produced by "reflection" or "mirroring" of the high-frequency portion of the pattern about the predicted isotropic chemical shift, as discussed previously.[17a] The positions of the three discontinuities in each half of the Pake doublet (i.e., the "foot", "shoulder" and "horn") depend directly on the values of C_Q and η_Q. Analytical simulations of idealized ^{14}N powder patterns were performed with the WSOLIDS software package.[23]

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Results and Discussion

General observations. We begin this analysis with a comparison of ^{14}N UW SSNMR spectra acquired with direct excitation followed by the WURST–CPMG pulse sequence, against sets collected with cross polarization by means of the BRAIN–CP/WURST–CPMG pulse sequence. For brevity, we designate these experiments and corresponding spectra as direct excitation (DE) and broadband cross polarization (BBCP), respectively. Close examination of the BBCP sequence reveals two distinct sections (Scheme 1). The BRAIN–CP section of the pulse sequence features a 90° pulse on the ^{1}H channel followed by a spin-locking pulse, exactly as in a conventional CP experiment. On the X channel, by contrast, the counterpart contact pulse is an amplitude- and phase-modulated WURST 80 pulse[13] (labeled "WURST A"), which by linearly sweeping over a range of frequency offsets serves to (i) fulfill the Hartmann–Hahn polarization transfer conditions sequentially over a broadband frequency range, and (ii) to "lock" the enhanced X spin-packets after their polarization transfer has been affected, performing on them an effective adiabatic sweep that ultimately enables their storage along the z direction at the end of the pulse. The portion of the sequence that follows utilizes the "WURST B" pulse to rotate this spin polarization from the z axis to the transverse plane, in which it can be detected and/or further manipulated. A train of "WURST C" pulses act as a CPMG-like train, that refocuses the dephasing of these severely inhomogeneously broadened resonances to yield a time-domain FID consisting of spin echoes, the length of which depends on the effective T_2 of the nucleus under observation. In all cases, relatively low ^{1}H power levels during the contact time were used to achieve efficient BBCP. However, comparatively higher ^{1}H decoupling fields (ca. 40 kHz) were used and found to be necessary[16b] for reducing the contribution of ^{1}H–^{14}N dipolar relaxation to the effective $T_2(^{14}N)$, thereby increasing the number of echoes in the CPMG echo train and maximizing the S/N (see Table S2 for details). Interestingly, the low rf fields available on the X (^{14}N) channel did not hinder us from achieving the Hartmann–Hahn matching conditions, owing to the increase in nutation rates endowed by the frequency-swept WURST A pulse.

In order to make quantitative S/N assessments, we compare single sub-spectra acquired with DE and BBCP methods featuring the same number of scans and identical experimental parameters in the WURST–CPMG portions of the pulse sequences. Single sub-spectra are used for these comparisons as opposed to the full- or half-Pake patterns, because the acquisition of the latter with DE methods is normally prohibitive in terms of long experimental times for most samples (vide infra).

Because full breadths of the patterns cannot be uniformly excited in a single experiment (even with broadband DE or CP pulses), multiple experiments conducted at uniformly spaced transmitter frequencies were used to acquire each pattern in its entirety. Important factors in designing and executing these experiments include the influence of the overlapping satellite transition patterns on the mechanism of CP and the ability to maintain a ^{14}N spin-lock and ensuring adiabatic broadband polarization transfer—two considerations unique to integer spin nuclei. For all of the spectra acquired with BRAIN–CP, the sweep bandwidths of all of the WURST pulses and the transmitter positions were chosen such that only one of the two transitions for each crystallite orientation was within the range of the inversion sweep, and that transmitter frequencies near the centre of the Pake doublet were avoided. This scheme is simple to enact under the assumption that the MHz-broad ^{14}N powder patterns are largely influenced only by the quadrupolar interaction to first order, with negligible effects from the second-order quadrupolar interaction and chemical shift anisotropy. Under this approximation, a single transition for each crystallite orientation is observed by recording sub-spectra with pulses swept towards the centre of the pattern (0 ppm), but without exceeding 0 ppm (crossing over to the negative ppm range). Because frequency-swept pulses do not yield complete population inversion near the edges of their sweep ranges, the polarization transfer at the centre of the powder pattern is reduced.

α-Glycine. α-Glycine, the most common polymorph of glycine, features a single crystallographically unique nitrogen site within an RNH_3^+ moiety.[24] It is an ideal starting point for comparison of UW SSNMR spectra acquired with DE and CP methods, because its $T_1(^{1}H)$ and $T_1(^{14}N)$ values are both small, allowing for short recycle delays (1 s) for each class of experiment. The ^{14}N SSNMR spectra acquired with DE and BBCP methods are compared in Figure 1A. These patterns are approximately 1.8 MHz wide (C_Q = 1.19(2) MHz and $\eta_Q = 0.52(2)$, in agreement with previous measurements),[4a,8b,25] and have been acquired with identical conditions in the WURST–CPMG portions of the respective sequences (i.e., the same parameters, including the number of scans, echoes, sub-spectra and spikelet spacings, see Tables S1 and S2). It is important to note that twenty sub-spectra were collected to construct both the DE and BBCP spectra, because the broadband CP excitation profile yielded by the BRAIN–CP sequence is comparable to that of the DE WURST echoes.

The BBCP spectrum has a S/N approximately five times higher than that of the DE spectrum (Table 1), which means that under these same experimental conditions, it would take approximately twenty-five times longer to acquire a DE spectrum of comparable S/N. A quantitative appraisal of the BBCP signal enhancement is made by comparing the S/N of five spikelets from sub-spectra collected near the "horn" of the powder pattern (Figure 1B). This is a dramatic enhancement, but it is well under the maximum theoretical value for the signal enhancement of approximately 13.8 times (i.e., $\gamma(^{1}H)/\gamma(^{14}N)$) that should be possible in systems with better CP characteristics (e.g., polarization transfer rate is faster, coherence lifetimes are longer, etc.). The BBCP spectrum is also richer in information than the DE spectrum. In particular, the outermost discontinuities, or the

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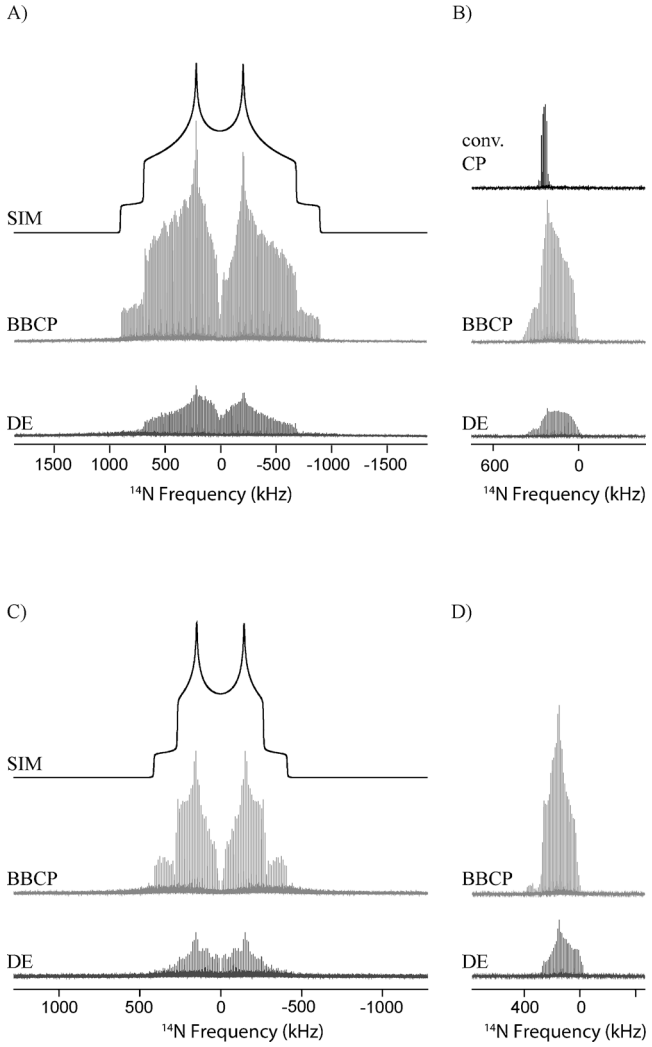

Figure 1. A) Full ^{14}N UW SSNMR spectra of glycine acquired with WURST–CPMG (DE) and BRAIN–CP/WURST–CPMG (BBCP) methods, presented together with an idealized analytical simulation (SIM). B) Single ^{14}N SSNMR sub-spectra of glycine acquired with an equal number of scans and WURST–CPMG parameters at a transmitter ^{14}N Larmor frequency of 29.045 MHz by DE, BBCP and conventional CP methods; note the narrower excitation bandwidth resulting from conventional CP when compared to the sub-spectrum acquired with BBCP. C) ^{14}N UW SSNMR spectra of trigonelline HCl acquired with DE and BBCP methods. Only the high-frequency half of the pattern was acquired, with the total Pake doublet formed by "reflection" of this pattern about the isotropic shift. D) Single ^{14}N SSNMR DE and BBCP sub-spectra of trigonelline HCl acquired at a transmitter frequency of 29.045 MHz and identical WURST–CPMG parameters.

"feet" of the powder pattern, are clearly observed in the BBCP spectrum, allowing for accurate spectral fitting of this region, which is not possible with the DE spectrum. The excitation bandwidth of BRAIN–CP is comparable to that of the WURST–CPMG experiment, and far superior to conventional CP (Figure 1B); in this case, frequency-stepped CP experiments would require the collection of approximately 3–5 times as many sub-spectra.

Trigonelline HCl. Trigonelline HCl features an sp^2 nitrogen site in an aromatic heterocycle. The ^{14}N SSNMR spectra acquired with DE and BBCP methods reveal spectra that are approximately 800 kHz wide ($C_Q = 0.55(2)$ MHz, $\eta_Q = 0.30(2)$, Figure 1C). Despite the narrowness of these patterns compared to those of glycine, the experimental times associated with their acquisition are much lengthier, due to the shorter $T_2(^{14}N)$ values for trigonelline HCl, as well as the longer $T_1(^{14}N)$ (in the case of DE experiments) and $T_1(^{1}H)$ (in the case of BBCP experiments) values. As discussed in the experimental section, only one half of the overall Pake doublet is acquired in both the DE and BBCP experiments, with the total Pake doublet produced by "reflection" or "mirroring" (this is more or less a cosmetic effect, due to the mirror symmetry of the pattern when the first-order quadrupolar interaction is dominant). The DE spectrum is comprised of four sub-spectra, each of which took two hours to acquire, for a total experimental time of eight hours. The BBCP spectrum, which has significantly higher S/N, is also comprised of four sub-spectra, each of which took only 21 min to acquire. The BBCP powder pattern clearly reveals the horn, shoulder and foot discontinuities, whereas the S/N of the DE spectrum is too low to allow extraction of the quadrupolar parameters because only one of the three characteristic discontinuities is observed. Comparison of single BBCP and DE sub-spectra collected with the same number of scans (Figure 1D) reveals that the former has an approximately fourfold improvement S/N over the latter. The BBCP experiment further benefits from the comparatively shorter recycle time that may be employed (15 s for BBCP vs. 45 s for DE). If both of these factors are taken into account, the DE experiment would have to be run for approximately 25 h to obtain comparable S/N to the CP spectrum (i.e., a sixteen-fold increase in the number of scans, each requiring three times longer to acquire).

Isoxsuprine HCl. Isoxsuprine HCl features an sp^3 nitrogen in an $R_2NH_2^+$ environment. The DE and BBCP ^{14}N SSNMR spectra are approximately 1.5 MHz wide, with quadrupolar parameters of $C_Q = 0.97(2)$ MHz and $\eta_Q = 0.75(5)$ (Figure 2A). This is the only "high η_Q" pattern (i.e., η_Q is near 1, meaning that $|V_{22}| \approx |V_{33}|$) discussed in the current work, where the two "horns" are close to one another near the centre of the spectrum (the high η_Q is typical for this type of nitrogen environment). The DE spectrum is comprised of five sub-spectra acquired in 200 kHz steps, taking approximately 60 h to acquire. By contrast, the total CP spectrum required the acquisition of seven sub-spectra in 90 kHz steps, with a total experimental time of only 5 h. The CPMG echoes in the FID of the DE experiment were closely spaced in order to maximize S/N, which leads to wider spacing of the spikelets in the frequency domain spectrum. This is in contrast to the BBCP experiment, which yields a much higher resolution spectrum in a fraction of the time. In fact, the BBCP spectrum was acquired approximately ten times faster than the DE spectrum, despite the smaller CPMG enhancement employed. For a quantitative comparison of S/N differences, the single sub-spectra acquired with the same number of scans (Figure 2B) and iden-

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Table 1. Comparisons of S/N enhancements in single ^{14}N SSNMR sub-spectra acquired with BRAIN–CP/WURST–CPMG (BBCP) and WURST–CPMG (DE) methods.

Compound	ν_{Tx}[a] [MHz]	Acquisitions	Recycle delay [s][b]	Experimental time [min]	S/N Ratio (BBCP:DE)[c]
Glycine	29.045	128	BBCP: 5 DE: 5	BBCP: 11 DE: 11	4.8
Trigonelline HCl	29.045	480	BBCP: 15 DE: 45	BBCP: 120 DE: 360	3.9
Isoxsuprine HCl	29.005	8640	BBCP: 2.5 DE: 2.5	BBCP: 360 DE: 360	6.3
Buflomedil HCl	29.405	1792	BBCP: 5 DE: 10	BBCP: 150 DE: 300	9.7

[a] ν_{Tx} denotes the transmitter frequency on the ^{14}N channel. The transmitter frequency was chosen such that it was proximate to the "horn" discontinuity of the ^{14}N powder pattern. [b] For BBCP and DE experiments, recycle delays are estimated as $5\times T_1(^1H)$ and $5\times T_1(^{14}N)$, respectively. For a complete list of acquisition parameters, see Table S2 in the Supporting Information. [c] The ratio of S/N values compares the average S/N obtained from BBCP and DE experiments. The average S/N values from BBCP and DE spectra are determined by measuring the S/N of five individual spikelets (phase corrected) in the proximity of the transmitter frequency.

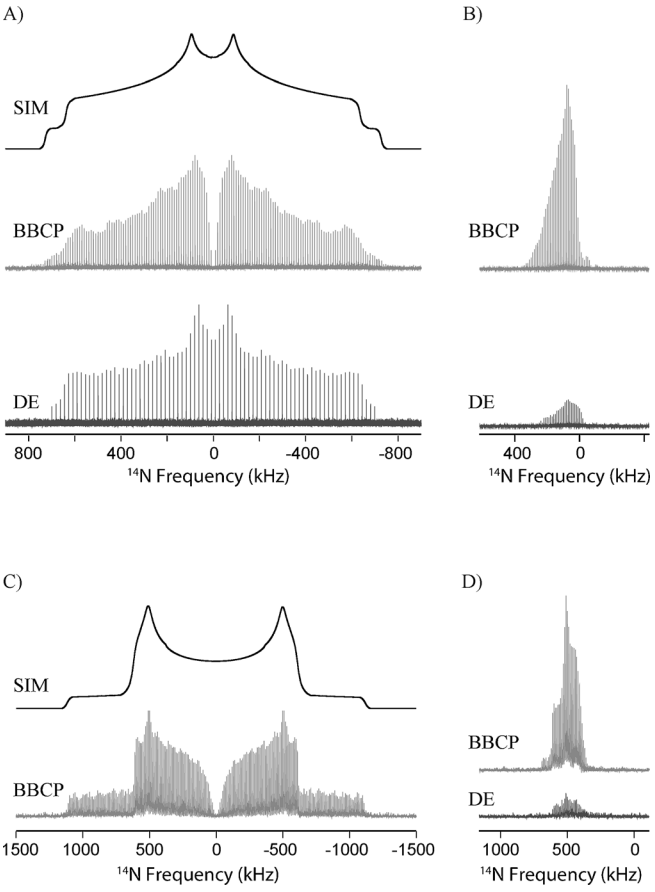

Figure 2. A) ^{14}N UW SSNMR spectra of isoxsuprine HCl acquired with DE and BBCP methods (high-frequency portion reflected), along with an analytical simulation. B) Single ^{14}N SSNMR DE and BBCP sub-spectra of isoxsuprine HCl acquired at a transmitter frequency of 29.005 MHz with the same number of scans and identical WURST–CPMG parameters. C) ^{14}N UW SSNMR spectra of buflomedil HCl acquired with BBCP (high-frequency portion reflected), along with an analytical simulation. D) Single ^{14}N SSNMR DE and BBCP sub-spectra of buflomedil HCl acquired with the same number of scans and identical WURST–CPMG parameters at a transmitter frequency of 29.405 MHz.

tical conditions in the WURST–CPMG portion of the pulse sequence reveal that the BBCP method is much more efficient, yielding a spectrum with approximately six times higher S/N than the corresponding DE spectrum.

Buflomedil HCl. Buflomedil HCl features an sp^3 nitrogen environment where the N atom is part of a saturated five-membered ring. All attempts with DE ^{14}N SSNMR experiments, including those employing WURST–CPMG echo trains, failed to produce sub-spectra with sufficient S/N to construct a complete powder pattern in reasonable experimental times. However, a ^{14}N SSNMR spectrum of high quality was acquired with the BBCP method (Figure 2C), revealing a pattern with a breadth of 2.2 MHz, and quadrupolar parameters of $C_Q=$ 1.49(2) MHz and $\eta_Q=0.10(3)$. The high-frequency half of this extremely broad spectrum is constructed from 12 sub-spectra acquired in 90 kHz steps over a total experimental time of 12 h, and the total pattern is generated by reflection. This spectrum was more difficult to acquire than the others discussed herein, due to the large breadth of the pattern as well as the reduced value of $T_2(^{14}N)$, which reduces the effectiveness of the CPMG enhancement; nonetheless, the positions of the outer discontinuities allow for the accurate extraction of quadrupolar parameters. The extremely low S/N of a single DE sub-spectrum (Figure 2D) reflects these difficulties; comparison of this single sub-spectrum to the BRAIN–CP sub-spectrum acquired at the same transmitter frequency reveals approximately ten times the S/N in the latter. The extreme breadth of the spectrum of buflomedil HCl shown in Figure 2C is clear evidence of the extraordinary sensitivity of the ^{14}N nucleus to its chemical environment, as well as the potential of the BRAIN–CP/WURST–CPMG method for the acquisition of ^{14}N SSNMR spectra that would otherwise be intractable.

Conclusion

We have demonstrated that it is possible to obtain high-quality ^{14}N UW SSNMR spectra of polycrystalline samples by combining the BRAIN–CP and WURST–CPMG pulse sequences to access the combined benefits of broadband cross polarization and broadband echo-train acquisition, respectively. A comparison of DE and BBCP spectra clearly indicates that the latter are superior in terms of S/N, reduced experimental times and spectral appearance. The only exception may be in rare instances in which the ^{1}H and ^{14}N relaxation conditions (in particular, a reduced $T_1(^{14}N)$), support a faster DE experiment. The ability to use lower Hartmann–Hahn matching fields for BRAIN–CP than would be required for conventional CP is also appealing, as this reduces the probe duty cycle and limits the occurrences of arcing and/or probe damage. Finally, the experiments are facile to set up and execute, with parameters that are similar

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in many respects to conventional CP experiments (versions of these pulse sequences for Bruker and Varian spectrometers are available from the corresponding author).

This work only scratches the surface of what can be done with ^{1}H–^{14}N broadband CP NMR experiments. The four distinct nitrogen structural moieties discussed herein are common to many organic and biological systems. This suggests that these methods can be extended to the study of variety of nitrogen environments associated with a large range of quadrupolar coupling constants. For ^{14}N spectra with multiple patterns possessing similar quadrupolar parameters, it might be possible to use both variable-contact time experiments and "T_2 editing" of the CPMG echo trains to differentiate between these patterns. This is because ^{14}N nuclei in different structural environments have distinct CP efficiencies and dissimilar effective transverse relaxation times. In the case of RNH_3^+ and $R_2NH_2^+$ moieties, variable-temperature BRAIN–CP experiments may also be used to investigate dynamic processes (much like ^{2}H SSNMR), as well as for further signal enhancement and site differentiation (by means of alteration of effective T_2 values and/or CP efficiencies). Finally, we stress that the experiments discussed herein were all conducted at 9.4 T and the use of ultra-high fields (e.g., 18.8 T or greater) will further increase the efficiency of these experiments. Given all of these possibilities, we believe that ^{1}H–^{14}N BRAIN–CP/WURST–CPMG NMR experiments will find widespread use in probing the structures of nitrogen-containing systems at the molecular-level in many areas of chemistry and biochemistry. This is especially likely, given the extreme sensitivity of ^{14}N NMR spectra to nitrogen chemical environments.

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