Proton chemical shift tensors determined by 3D ultrafast MAS double-quantum NMR spectroscopy

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Proton NMR spectroscopy in the solid state has recently attracted much attention owing to the significant enhancement in spectral resolution afforded by the remarkable advances in ultrafast magic angle spinning (MAS) capabilities. In particular, proton chemical shift anisotropy (CSA) has become an important tool for obtaining specific insights into inter/intra-molecular hydrogen bonding. However, even at the highest currently feasible spinning frequencies (110–120 kHz), ^1H MAS NMR spectra of rigid solids still suffer from poor resolution and severe peak overlap caused by the strong ^1H – ^1H homonuclear dipolar couplings and narrow ^1H chemical shift (CS) ranges, which render it difficult to determine the CSA of specific proton sites in the standard CSA/single-quantum (SQ) chemical shift correlation experiment. Herein, we propose a three-dimensional (3D) ^1H double-quantum (DQ) chemical shift/CSA/SQ chemical shift correlation experiment to extract the CS tensors of proton sites whose signals are not well resolved along the single-quantum chemical shift dimension. As extracted from the 3D spectrum, the F1/F3 (DQ/SQ) projection provides valuable information about ^1H – ^1H proximities, which might also reveal the hydrogen-bonding connectivities. In addition, the F2/F3 (CSA/SQ) correlation spectrum, which is similar to the regular 2D CSA/SQ correlation experiment, yields chemical shift anisotropic line shapes at different isotropic chemical shifts. More importantly, since the F2/F1 (CSA/DQ) spectrum correlates the CSA with the DQ signal induced by two neighboring proton sites, the CSA spectrum sliced at a specific DQ chemical shift position contains the CSA information of two neighboring spins indicated by the DQ chemical shift. If these two spins have different CS tensors, both tensors can be extracted by numerical fitting. We believe that this robust and elegant single-channel proton-based 3D experiment provides useful atomistic-level structural and dynamical information for a variety of solid systems that possess high proton density. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4933114>]

I. INTRODUCTION

Due to its capability of selectively manipulating different anisotropic spin interactions in solids, solid-state NMR (SS-NMR) spectroscopy has always been a versatile tool that can provide atomic-level insights into various molecular systems.^{1,2} Indeed, over the past decades, SSNMR has become a routine method for studying low- γ nuclei (^{13}C , ^{15}N , etc.) due to their large chemical shift (CS) spans.^{3,4} However, SSNMR often requires a large sample volume and long experimental time for achieving reasonable signal-to-noise ratio, unless sensitivity enhancement methods (e.g., dynamic nuclear polarization (DNP),^{5–7} parahydrogen induced polarization (PHIP),^{8,9} and optical pumping^{10,11}) are employed.⁵ Nonetheless, with the tremendous developments in magic angle spinning (MAS) probe design and technology that have recently enabled spinning up to 120 kHz, the proton spectral resolution can be significantly improved due to the effective suppression of strong anisotropic spin interactions that cause line broadening in ^1H NMR spectra of solids.^{12–16} Combined with high magnetic fields to enhance spectral resolution and sensitivity, proton-based SSNMR experiments have become

an attractive choice for investigating the molecular structure and dynamics in a wide range of systems, including proteins,^{17–22} molecular pharmaceuticals,^{23–25} polymers,^{26–28} biomaterials,^{12,29} and other systems. In particular, there has been considerable interest in measuring the proton CS tensors^{30–33} due to their sensitivity to local inter- and intramolecular interactions, such as hydrogen bonding and ring current effects, which play important roles in controlling the three-dimensional (3D) molecular conformation and packing in solid-state structures of an exciting class of organic and functional materials. Indeed, various methods have been proposed to extract the chemical shift tensors of protons,^{34–38} among which is the recently introduced simple 2D chemical shift anisotropy/single-quantum (i.e., isotropic chemical shift) (CSA/SQ) correlation experiment that has gained popularity in extracting CSA tensors, as well as determining relative CSA orientations due to the improved spectral resolution enabled by ultrafast MAS.^{37,39}

In spite of the improved proton spectral resolution by ultrafast MAS and high magnetic fields, the ^1H -detected NMR spectra of dense proton systems still suffer from severe overlap of proton spectral lines. In this case, the anisotropic CS line shapes obtained by extracting the relevant slices at certain isotropic chemical shifts generally contain information on the anisotropy and asymmetry of multiple proton sites with

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different chemical environments. Herein, we propose a simple and elegant 3D double-quantum (DQ)/CSA/SQ correlation experiment that could correlate the DQ signal with CSA evolution in a robust manner. As the proton spectrum along the DQ dimension has a better resolution than the SQ dimension due to the doubled spectral width in the former dimension, the correlation of CSA and DQ enables the precise measurement of chemical shift tensors for protons, particularly for those that give rise to overlapping and poorly resolved resonances in the isotropic chemical shift SQ dimension.

II. EXPERIMENTS

A. Materials

Ibuprofen was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification.

B. Solid-state NMR spectroscopy

Solid-state NMR experiments were performed on an Agilent VNMRS 600 MHz NMR spectrometer operating at a ^1H frequency of 599.8 MHz and equipped with a triple-resonance 1.2-mm MAS probe. The 3D DQ/CSA/SQ pulse sequence used in this study is shown in Figure 1. Rotor-synchronized broadband back-to-back (BABA)⁴⁰ sequence was employed for the DQ excitation and reconversion, and a composite- 180° (namely, $270^\circ_0\text{--}90^\circ_{180}$)-pulse-based symmetry sequence $\text{R}18_8^7$ was utilized for recoupling proton chemical shift interactions immediately after the DQ reconversion period.³⁷ Before the final 90° read pulse, a short delay of ~ 0.5 ms was introduced to filter out the residual proton transverse magnetization. The single-channel R-symmetry-based CSA recoupling sequence also recouples the heteronuclear dipolar couplings, because both heteronuclear dipolar coupling and chemical shift anisotropy tensor have a space rank of 2 and a spin rank of 1.⁴¹ Thus, the CSA line shapes for the nitrogen-bonded protons might be affected by the recoupled $^1\text{H}\text{--}^{14}\text{N}$ dipolar

couplings unless ^{14}N decoupling is achieved. According to the symmetry principle, if a rectangle 180° pulse is adapted, the π pulse length in the $\text{R}18_8^7$ sequence must be set to $l_\pi = \frac{4\tau_R}{9}$, where τ_R is the rotor period. When a composite 180° pulse (i.e., $270^\circ_0\text{--}90^\circ_{180}$) is used, the π pulse length is $l_\pi = \frac{2\tau_R}{9}$. Therefore, the RF strength is $w_1 = \frac{9w_R}{4}$, where w_R is the spinning frequency. This means that higher spinning frequencies require stronger RF field strengths. As recently reported,³⁷ faster spinning rate also helps in suppressing the proton homonuclear dipolar couplings and thus enables high CSA recoupling efficiency. Overall, high spinning speeds are preferred for the CSA recoupling experiment due to their efficiency in the suppression of dipolar couplings and enhancement of spectral resolution. The experiment in this study was performed at a MAS rate of 61.728 kHz with a ^1H 90° pulse length of $1.8\ \mu\text{s}$. The DQ excitation/reconversion time was around $64.8\ \mu\text{s}$. 48 complex t_1 points, 24 real t_2 points, and 256 complex t_3 points were acquired with dwell times of 15.9, 129.6, and $20\ \mu\text{s}$, respectively. 8 transients per increment were co-added with a recycle delay of 3 s. The data were zero-filled to yield a $1024 \times 128 \times 256$ data matrix and were processed in NMRPipe.⁴² The 2D spectra were directly obtained from the skyline projections of the 3D spectrum.

C. Numerical simulations

Numerical simulations of ^1H CSA powder line shapes were performed using the SIMPSON software package,^{43,44} in which the CS tensor is defined by three parameters: the isotropic chemical shift $\delta_{\text{iso}} = (\delta_{xx} + \delta_{yy} + \delta_{zz})/3$, the CSA $\delta_{\text{aniso}} = |\delta_{zz} - \delta_{\text{iso}}|$, and the asymmetry parameter $\eta = (\delta_{yy} - \delta_{xx})/\delta_{\text{aniso}}$. The principal components (δ_{xx} , δ_{yy} , and δ_{zz}) of the chemical shift tensor are ordered according to $|\delta_{zz} - \delta_{\text{iso}}| \geq |\delta_{xx} - \delta_{\text{iso}}| \geq |\delta_{yy} - \delta_{\text{iso}}|$. Powder averaging for the R -type symmetry sequence was performed using either 168 or 678 pairs of (α, β) angles selected according to the REPULSION⁴⁵ scheme, and 26 γ angles. To extract accurate best-fit CS parameters, all parameters used in simulations were

FIG. 1. Pulse sequence used for the 3D DQ/CSA/SQ correlation experiment. Broadband BABA sequence is utilized for DQ excitation and reconversion, while a composite- 180° (namely, $270^\circ_0\text{--}90^\circ_{180}$)-based symmetry sequence $\text{R}18_8^7$ is used for recoupling proton CSA. The black solid rectangle in the broadband BABA sequence indicates a 90° pulse. In the $\text{R}18_8^7$ sequence, the phase-alternating pulses are indicated with solid (black and grey) and blank (black and grey) rectangles with phases as indicated.

FIG. 2. (a) Molecular structure of ibuprofen and the proton labeling scheme used in this study. (b) 2D DQ/SQ (F1/F3) chemical shift correlation spectrum extracted from the 3D spectrum. The blue lines indicate the DQ correlations produced by different proton spins. The 1D ^1H NMR spectrum of ibuprofen at 61.7 kHz MAS is shown above the 2D spectrum along with the corresponding proton assignments.

set identical to their experimental values. The best-fit curves were obtained using the newly developed *opt* package based on the SIMPLEX optimization routine, which is part of the VMD molecular visualization program.⁴⁶ Because the zero-frequency peaks generally do not contain useful information, fitting of these peaks was not included in the line shape simulations. The CSA splitting was obtained by directly measuring the frequency difference between the two singularities in the CSA line shape.

III. RESULTS AND DISCUSSION

Ibuprofen is a commonly used anti-inflammatory drug with a well-characterized crystal structure (Fig. 2(a)).^{47,48} It is used in this study as a model system to demonstrate the

performance of the pulse sequence, the results of which are shown in Figures 2-4. Indeed, this single-channel proton-based 3D sequence provides ample information about the molecular structure in terms of dipolar couplings and chemical shift anisotropy. Three different 2D spectra, including DQ/SQ (F1/F3), CSA/SQ (F2/F3), and CSA/DQ (F2/F1) correlations, could be extracted from a single 3D spectrum.

The molecular structure of ibuprofen is shown in Fig. 2(a), and the DQ/SQ correlation spectrum extracted from the 3D spectrum is shown in Fig. 2(b). Within a DQ excitation/reconversion time of 64.8 μs , a spin pair in close proximity generates a DQ signal, giving rise to a pair of peaks with the same chemical shift along the DQ dimension, which is the sum of the respective isotropic chemical shifts of the two different resonances along the SQ dimension. Thus, this DQ/SQ chemical shift correlation spectrum provides important information about the proximity of various protons within the molecular framework. For example, the pair of cross-peaks appearing at ~ 13.4 ppm in the DQ dimension arise from the correlation between the peaks at ~ 12.6 and ~ 0.8 ppm in the SQ dimension; and they must result from the dipolar coupling between the OH (H1) and the nearby CH₃ (H11) protons that are ~ 4.36 Å away,⁴⁷ and not due to the H1-H9 or H1-H10 dipolar couplings because both the H1-H9/H10 intra- and intermolecular separations are extremely large to be detected in a DQ excitation/reconversion period of 64.8 μs . According to the neutron-diffraction crystal structure of ibuprofen,⁴⁷ the OH proton (H1) lies ~ 8.2 Å away from the closest CH₃ protons of the isobutyl moiety (H9 and H10) in the same ibuprofen molecule. At a DQ excitation/reconversion time of 64.8 μs , it is therefore too weak to observe the DQ signals arising from correlations between proton 1 and proton 9 (or 10) of the same molecule.

In addition, the CSA/SQ (F2/F3) correlation spectrum extracted from the 3D spectrum reveals different chemical shift environments of protons (Fig. 3(a)), and the chemical shift tensors are extracted by fitting the slices at the specific isotropic chemical shifts, as shown in Fig. 3(b). The experimental chemical shift parameters extracted by SIMPSON numerical simulations are listed in Table I. It is obvious that the chemical shift anisotropy δ_{aniso} of 14.8 ± 0.5 ppm for the -OH proton is much larger than that of other protons in ibuprofen, with the other protons all having δ_{aniso} of about 6.0 ± 0.4 ppm. The larger δ_{aniso} for the -OH proton is, to a large degree, due to the effect of

FIG. 3. (a) 2D CSA/SQ correlation spectrum extracted from the 3D spectrum of ibuprofen. (b) The CSA slices at different isotropic chemical shifts are shown in black, along with the corresponding simulated line shapes shown in red.

FIG. 4. (a) 2D CSA/DQ correlation spectrum extracted from the 3D spectrum of ibuprofen. The CSA slice at the DQ frequency of 19.7 ppm (b), and that at 13.4 ppm (c). Experimental line shapes are indicated in black, while the simulated ones are indicated in red and blue.

intermolecular hydrogen bonding interactions.^{25,47,49} Indeed, this can be readily understood by realizing that crystalline ibuprofen exists in a dimeric form, in which two independent ibuprofen molecules are joined together by two H-bonds between their carboxylic $-\text{COOH}$ groups.^{47,48}

Signals from the aliphatic CH/CH_2 (6,7,8) and CH_3 (9,10,11) protons are overlapped in the regions around 2.3 and 0.8 ppm, respectively. Therefore, the chemical shift parameters extracted from the CSA slices at the isotropic chemical shift of 2.3 and 0.8 ppm represent the overall/superimposed chemical shift environments of all these protons; hence, it might not accurately describe the chemical shift environment of the specific protons. However, by taking advantage of the high spectral resolution in the DQ dimension, the CSA/DQ correlation spectrum can be used to extract the chemical shift tensors of protons that give rise to overlapped resonances in the single quantum dimension, as shown in Fig. 4.

The advantage of the DQ dimension is its doubled spectral width in comparison to the SQ dimension, thus enabling a much better proton spectral resolution. Hence, the 2D CSA/DQ correlation spectrum, extracted from the 3D spectrum (Fig. 4(a)), makes it possible to determine the CS tensors of protons whose signals are overlapped in the SQ chemical shift dimension. Indeed, the CSA slices at a specific chemical shift in the DQ dimension contain information about the CSAs of both proton sites that are sufficiently close to produce the DQ signal. As is shown in Fig. 2 and discussed above, the resonances at 19.7 ppm and 13.4 ppm in the DQ dimension represent the hydroxyl/aromatic and hydroxyl/methyl (H11)

proton correlations, and their corresponding CSA slices are shown in Figs. 4(b) and 4(c), respectively. It is apparent from Figs. 4(b) and 4(c) that two pairs of singularities can be distinguished in both CSA line shapes indicating the presence of two distinct CS tensors. For obtaining accurate best-fit results, we have excluded the numerical fitting of the zero-frequency peak that generally results from RF field inhomogeneity, amplitude/phase transient effects, and/or sample spinning fluctuations.³⁷ Additionally, as the line shapes suffer from some baseline distortions or noises, these noisy/distorted portions were also excluded from numerical fittings. Only the two pairs of singularities were included in numerical simulations of the CSA line shapes (a detailed argument underlying the validity of this choice is discussed below).

In fitting the outside pair of singularities, we have considered the (0.75–1.5 kHz) and (–1.5 to –0.75 kHz) spectral ranges, while the (0.1–0.4 kHz) and (–0.4 to –0.1 kHz) spectral ranges were considered for fitting the inside pair of singularities. For the CSA line shapes sliced at 19.7 ppm in the DQ dimension, the outside pair of singularities corresponds to the hydroxyl protons, while the inside pair of singularities corresponds to the aromatic protons. The iterative numerical fitting gives ($\delta_{\text{aniso}} = 14.7 \pm 0.3$ ppm, $\eta = 0.4 \pm 0.2$) for the hydroxyl proton and ($\delta_{\text{aniso}} = 5.7 \pm 0.4$ ppm, $\eta = 0.7 \pm 0.3$) for the aromatic protons. Both results are in good agreement with the parameters extracted from the CSA/SQ correlation spectrum shown in Table I. Moreover, iterative numerical fitting of the CSA line shapes sliced at 13.4 ppm in the DQ dimension gives ($\delta_{\text{aniso}} = 13.7 \pm 0.6$ ppm, $\eta = 0.4 \pm 0.2$) for the hydroxyl proton and ($\delta_{\text{aniso}} = 4.9 \pm 0.4$ ppm, $\eta = 0.4 \pm 0.3$) for the methyl protons close to the hydroxyl group. The chemical shift parameters extracted here are also in line with the parameters obtained from the CSA/SQ correlation experiment. It is noteworthy that the chemical shift parameters for the methyl group close to the hydroxyl group could be successfully extracted from this CSA/DQ correlation spectrum, which is not possible from the regular 2D CSA/CS correlation experiment due to signal overlap. Taken together, these results demonstrate the robust performance of our 3D pulse sequence by correlating CSA and DQ to determine the chemical shift tensors of

TABLE I. ^1H chemical shift parameters extracted from numerical fittings of experimental line shapes shown in Figure 3.

	δ_{iso} (ppm)	δ_{aniso} (ppm)	η
–OH	12.6	14.8 ± 0.5	0.4 ± 0.2
Aromatic –CH	7.1	6.0 ± 0.4	0.7 ± 0.3
Aliphatic CH/CH_2	2.3	6.6 ± 0.6	0.6 ± 0.4
CH_3	0.8	5.5 ± 0.5	0.6 ± 0.4

FIG. 5. Numerical simulations of CSA line shapes with different values for the chemical shift anisotropy parameter δ_{aniso} and asymmetry parameter η at a MAS rate of 61.728 kHz. (a) Simulated CSA line shapes of variable η with $\delta_{\text{aniso}} = 15$ ppm. (b) Simulated CSA splitting as a function η with different δ_{aniso} values, as indicated. (c) Simulated CSA line shapes of variable δ_{aniso} with $\eta = 0.6$. (d) Simulated CSA splitting as a function δ_{aniso} with different η as indicated. The isotropic chemical shift was set to 0 ppm for all numerical simulations.

protons whose signals show significant overlap in the isotropic chemical shift dimension.

It is important to recall that the determination of experimental CS parameters relies completely on the numerical simulation of CSA line shapes. It is therefore necessary to understand the effect of the anisotropy δ_{aniso} and asymmetry η on the CSA line shapes and CSA splitting (i.e., the frequency difference between the two singularities), as shown in Fig. 5. At constant δ_{aniso} , the CSA splitting decreases with increasing η , as given in Figs. 5(a) and 5(b). With $\delta_{\text{aniso}} = 15$ ppm, the CSA splitting decreases from 2.1 kHz to 1.5 kHz, which is ~ 1 ppm at 14.1 T (600 MHz ^1H Larmor frequency). When δ_{aniso} is smaller (< 15 ppm), the decrease in CSA splitting becomes much less than 1 ppm with increasing η , as given in Fig. 5(b). To a large degree, one can conclude that the CSA splitting is insensitive to the asymmetry parameter. However, the line shapes around the singularities are found to vary significantly with η . Similarly, the line shapes around the singularities obviously differ with increasing δ_{aniso} , as given in Fig. 5(c). In particular, the CSA splitting increases linearly with increasing δ_{aniso} , as shown in Fig. 5(d). The CSA line shapes around the singularities are thus sensitive to both η and δ_{aniso} . However, the CSA splitting is quite insensitive to η , whereas it increases linearly with increasing δ_{aniso} . Therefore, performing the numerical simulation around the singularities is quite sufficient for obtaining accurate CSA parameters, as performed above.

The premise for determining accurate CS parameters is to record reliable and undistorted experimental powder CS line shapes. The phase-alternating composite- 180° -based R-type sequence used here, R18 $_8^7$ (270°_0 - 90°_{180}), has been demonstrated to overcome RF field inhomogeneity with a remarkable CSA recoupling efficiency.³⁷ On the other hand, the DQ excitation/reconversion time has to be optimized in order to achieve high DQ signal sensitivity, which is essential for obtaining

undistorted CS line shapes sliced at different DQ chemical shifts. In this study, we have utilized the broadband BABA⁴⁰ scheme for DQ excitation/reconversion, in which the minimum excitation/reconversion time is 4 rotor periods. If the sample is extremely rigid, the original BABA⁵⁰ sequence may be employed, using one rotor period as the minimum DQ excitation time; however, it might suffer from chemical-shift-offset effects at high magnetic fields. Conversely, if the system under investigation is relatively mobile, a super-cycle BABA-xy16⁵¹ is recommended for DQ excitation with a minimum excitation time of 8 rotor periods, which is robust enough to overcome the effects of RF inhomogeneity, chemical-shift offsets, and large chemical-shift anisotropies.

IV. CONCLUSION

In this study, we have experimentally demonstrated a 3D DQ/CSA/SQ pulse sequence that provides a reliable tool for site-specific ^1H CS measurements in solids under ultrafast MAS conditions, which offers the opportunity to obtain detailed information about molecular structures. The use of ultrafast MAS significantly suppresses all anisotropic interactions to render very high spectral resolution in the 3D spectrum, and proton detection enhances the sensitivity of the experiment despite the small amount of sample required by the rotor size. As demonstrated by the experimental results, the DQ/SQ (F1/F3) chemical-shift correlation spectrum provides insights into proton-proton proximities, while the CSA/SQ (F2/F3) correlation spectrum is used to extract the chemical shift tensors of protons at different sites. However, when the signals of specific protons are not sufficiently resolved, as in the case of most solid ^1H NMR line shapes, the extracted chemical shift parameters do not reflect the actual local environments of the specified protons. In this aspect, the CSA/DQ (F2/F1)

correlation spectrum demonstrates its advantage due to the enhanced spectral resolution in the DQ dimension. Although the powder line shape sliced at a specific DQ chemical shift contains CSA information of two different spin sites, the chemical shift parameters of both sites can still be successfully extracted as long as the chemical shift anisotropies of the two sites slightly differ from each other. Even though very fast sample spinning (up to 120 kHz), as well as spectral editing techniques and deuterium labeling, can dramatically increase the resolution of proton spectral lines by effectively suppressing the dipolar couplings among protons, magnetic susceptibility and conformational heterogeneity in some solid systems can still limit the spectral resolution by contributing to the observed broadening in proton linewidths. Therefore, it is hoped that this 3D sequence will be widely used for the measurement of accurate chemical shift parameters of protons whose isotropic chemical shift peaks are poorly resolved.

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