Solid-State NMR Spectroscopy

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Rapid Analysis of Organic Compounds by Proton-Detected Heteronuclear Correlation NMR Spectroscopy with 40 kHz Magic-Angle Spinning**

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High-resolution magic-angle spinning (MAS) solid-state NMR spectroscopy is a powerful tool for analyzing the structural and dynamical properties of organic compounds; for example, unique insights into solid-phase polymorphism/pseudopolymorphism, hydrogen bonding, and stereoisomerism have been achieved,[1,2] and these results apply to important classes of pharmaceutical solids in both pure and dosage forms.[3] Most studies have employed simple one-dimensional ^{13}C spectra, and resonances were assigned by comparison with solution-state spectra.[3,4] Unambiguous assignments are usually not possible when the solid and solution spectra differ substantially. Spectral editing experiments, which distinguish carbon types (C, CH, CH_2, CH_3), aid in confirming some assignments but are not always reliable, for example, when significant molecular dynamics are present.[3]

Two-dimensional techniques—as commonly employed for isotopically enriched materials in the solid state or at natural abundance in the solution state—greatly accelerate data interpretation on the basis of improved spectral resolution and information on rich-spin connectivity. For example, a ^{13}C–^{13}C refocused INADEQUATE spectrum of oxybuprocaine hydrochloride was utilized to assign resonances to two molecules in the asymmetric unit.[5] However, that experiment required five days of data collection and 80 mg of material, and is a fair representation of the sensitivity challenge presented by the low natural abundance of ^{13}C. Heteronuclear correlation (HETCOR) experiments involving ^{1}H and rare nuclei (^{13}C or ^{15}N) enhance sensitivity for resonance assignments (e.g., one day for ca. 20 mg of sample).[6,7] Inverse proton detection at 30-kHz MAS rate has been demonstrated on a polymer and a ^{13}C-labeled heptapeptide, with sensitivity enhanced approximately two- to threefold.[8] At even faster MAS rates (ca. 40 kHz) with probes optimized for ^{1}H sensitivity, we have shown further improvements in sensitivity and proton resolution.[9,10] Using the same method, Pruski and co-workers have also dramatically accelerated data collection for surface-bound molecules.[11]

Herein we apply proton-detected 40-kHz MAS HETCOR experiments on organic compounds for rapid data acquisition (30 min) with small sample quantities (ca. 6 mg, of which less than 4 mg is active pharmaceutical ingredient (API)). The reduced sample volume (8 μL) of 1.6-mm rotors is compensated by a high filling factor of a small sample coil, and the resulting sensitivity is comparable to that observed with much larger sample quantities.[9] In addition to facilitating proton detection, fast MAS results in spectra free of spinning side bands, which are easier to interpret; therefore, resonance assignments, polymorphism, dynamics, and hydrogen bonding are readily analyzed. Demonstrations herein include tablet formulations of ibuprofen and acetaminophen; using our recent solvent suppression technique MISSISSIPPI,[12] sufficient attenuation of water and other unwanted proton signals is achieved, so that the results will readily translate to hydrated formulations such as creams, ointments, and suspensions.

Ibuprofen, a widely used nonsteroidal anti-inflammatory drug, has a known three-dimensional crystal structure[13] and is frequently used as a model compound to study the impact of formulation on physical and chemical properties of the drug.[14–16] Its ^{1}H and ^{13}C resonance assignments were completed only recently with the utilization of ^{1}H–^{13}C HETCOR experiments, which have also been proven very powerful in discerning acid and sodium forms of ibuprofen.[14] However, prolonged data acquisition from three to six days was necessary for these ^{13}C-detected HETCOR experiments with more than 20 mg of sample.[14]

With the sensitivity gain offered by ^{1}H detection, we acquired a high-quality ^{13}C–^{1}H HETCOR spectrum (Figure 1 a) in 33 min on 6 mg of ibuprofen formulation containing less than 4 mg of the API. Excipients do not interfere with the API peaks, since the former are observed in the chemical shift range consistent with carbohydrates; the excipients also have a significantly longer T_1 relaxation times in this instance.[16] Signal-to-noise ratios (SNR, shown as average ± standard deviation) are 34 ± 22 (or 37 ± 22 without linear prediction) for all ibuprofen peaks and 46 ± 19 (or 48 ± 18 without linear prediction in the indirect dimension) for one- and two-bond correlation peaks.

This HETCOR spectrum facilitates straightforward assignments of all carbon and proton nuclei in ibuprofen, in detail as follows. The peak at (185.2, 12.7) ppm is assigned to the carboxy group (labeled a). Proton frequencies of b and c of 2.3 and 0.7 ppm, respectively, correspond to the nearby

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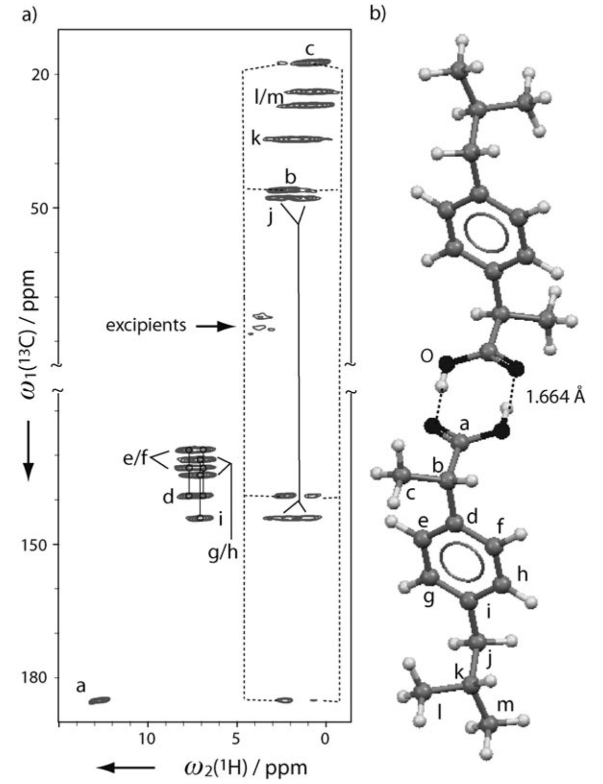

Figure 1. HETCOR experiment on dosage-form ibuprofen containing 3.8 mg of API. a) ^{13}C–^1H 2D spectrum acquired in 33 min, two scans per row, recycle delay 3 s, $t_{1max}(^{13}C) = 3.85$ ms, with forward linear prediction to 7.7 ms, $t_{2max}(^1H) = 5$ ms. Lorentzian-to-Gaussian apodization was applied (200 to 400 Hz for ^1H, 20 to 40 Hz for ^{13}C) followed by sine bell apodization (with 54° shift) for each dimension. Contours are drawn starting at six times the noise level, with a spacing factor of 1.4. b) Ibuprofen molecules showing labels and hydrogen-bond length across the dimer (Cambridge Structural Database code IBPRAC02).[13]

protons attached to the neighboring methine and methyl groups. Methine carbon atom b can then be located at 46.0 ppm. The peak at 47.8 ppm seems to have similar proton frequency, but close inspection of the data reveals that it consists of two broad components (ca. 1.3 and 2.7 ppm), consistent with the strongly coupled CH_2 group j. Upfield in the ^1H dimension from the strong peak b, a correlation to proton resonance c is also observed. Peaks with ω_1 at 17.3 and 23.9 ppm correlate to methyl ^1H resonances at about 0.7 ppm; the resonance with $\omega_1 = 17.3$ ppm is also coupled to $\omega_2 = 2.3$ ppm (^1H frequency b), that is, the peak at (17.3, 0.7) ppm is due to methyl group c. The two remaining methyl groups l and m are assigned to peaks at (26.9, 0.9) and (23.9, 0.7) ppm, and the methine group k is associated with (35.6, 1.8) ppm in accordance with the typical chemical shifts for tertiary carbon atoms. Aromatic quaternary carbon atom i at 144.1 ppm is identified with connectivity to proton j frequencies; carbon i also correlates to neighboring aromatic protons g and h as a single unresolved peak at (144.1, 7.0) ppm. Carbon atoms g and h are then located at 134.4 and 131.0 ppm based on the resemblance of their proton resonance position and lineshape to those of peak i. The other aromatic quaternary carbon atom d at 139.1 ppm is identified from the two correlation peaks with b and c proton frequencies; carbon atom d also correlates to neighboring aromatic protons e and f as two equally strong and partially resolved peaks at (139.1, 7.7) and (139.1, 6.9) ppm. Carbon resonances e and f are located at 132.7 and 128.7 ppm according to the same proton resonance doublet lineshape as for carbon atom d.

Important information on dynamics can also be extracted from the HETCOR spectrum. The aromatic-ring carbon atoms e (g) and f (h) are magnetically distinct, consistent with a rigid ring on the chemical-shift timescale (ca. 1 ms). Furthermore, carbon atom e (or f) correlates to both e and f proton frequencies with nearly equal peak intensities, consistent with an exchange process which is slow on the chemical-shift timescale, but faster than the timescale of longitudinal storage (270 ms) of ^{13}C magnetization during MISSISSIPPI suppression.[12,17] The possibility of millisecond exchange that may occur during evolution periods can be excluded by the lack of any significant motional broadening of the ^{13}C and ^1H resonances.[17] Methyl groups l and m have much broader linewidths than methyl group c (1300 versus 700 Hz, including 200 Hz applied broadening), which indicate that rotation around the threefold symmetry axis is more restricted for l and m than for c.

Another benefit of proton NMR is direct insight into hydrogen-bonding derived from the proton chemical shift.[1] For O−H···O hydrogen bonds, a linear correlation between the hydrogen-bond length $r_{H\cdots O}$ [Å] and the isotropic chemical shift δ [ppm] was demonstrated [Eq. (1)] using crystallographic and NMR data assembled by Jeffrey and Yeon.[18] Note that the equation is derived by the current authors from data in the literature.

$$r_{H\cdots O} = (2.210 \pm 0.005) - (0.044 \pm 0.004)\,\delta \qquad (1)$$

From this equation, $r(H\cdots O) = 1.65 \pm 0.06$ Å is calculated for the proton chemical shift of 12.7 ± 0.15 ppm, in agreement with the hydrogen-bonding distance of 1.664 ± 0.010 Å across the carboxylic acid dimer according to neutron diffraction (Figure 1 b).[13]

Among the three polymorphs of acetaminophen (i.e., paracetamol), the monoclinic form is chosen for drug formulation due to its thermodynamic stability.[19] The Raman spectra of the monoclinic and orthorhombic forms are identical, but differences are observed in the IR and ^{13}C NMR spectra.[20] Nevertheless, one-dimensional ^{13}C spectra did not allow aromatic carbon atoms to be specifically assigned,[20] and ^{13}C-detected HETCOR spectra were time-prohibitive due to the very long proton T_1 value (63 s) in this tablet formulation (in contrast with 1.7 s for the ibuprofen sample above). With ^1H detection, we acquired a HETCOR spectrum of adequate SNR (20 ± 8 excluding 177 for the strong methyl peak; or 21 ± 7 excluding 153 for the methyl peak without linear prediction in the indirect dimension) with only two scans per t_1 increment and a recycle delay of 35 s (Figure 2 a). In the scenario of multiple samples with long T_1 values, we envision combinations of high-sensitivity proton detection with probe designs incorporating multiple MAS modules.[21]

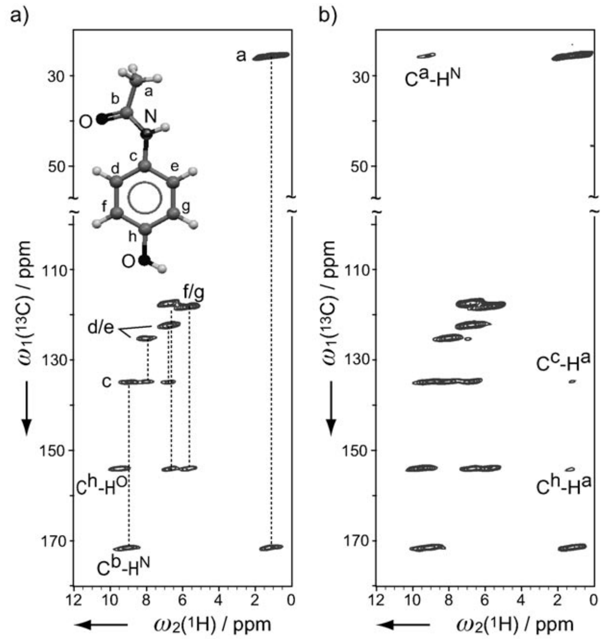

Figure 2. HETCOR experiment on dosage-form acetaminophen containing 5.5 mg of API. a) ^{13}C–^1H 2D spectrum acquired in 6 h with two scans per row, recycle delay 35 s. b) ^{13}C–^1H 2D spectrum acquired in 18 h with six scans per row. Other parameters are the same as in Figure 1. The inset of a) shows the molecule with labels.

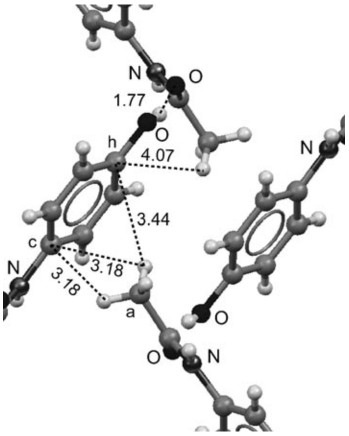

Figure 3. Monoclinic acetaminophen in a unit cell, viewed along the *a* axis; the *b* axis is horizontal, and the *c* axis vertical (Cambridge Structural Database code HXACAN07).[22] Distance values are given in Å.

Assignments of all carbon and proton nuclei in acetaminophen can also be readily made with the HETCOR spectrum. The peak at (25.6, 1.1) ppm is readily assigned to methyl group a. The resonance at 171.5 ppm is assigned to carbonyl carbon atom b, which can be confirmed by a cross-peak with the methyl proton. The amide proton is then assigned to 9.0 ppm from the cross-peak with the carbonyl carbon atom. Aromatic quaternary carbon atom c is found at 134.9 ppm with correlation to the amide proton as well as d and e protons at 8.0 and 6.7 ppm. Carbon resonances d and e are found at 134.9 and 122.3 ppm. Then the peak at (154.1, 9.5) ppm with low-field proton shift is assigned to the correlation between the other quaternary carbon atom h and the hydroxyl proton. Carbon atom h also correlates to two other protons f and g at 6.7 and 5.6 ppm. Carbon atoms f and g are then assigned to 117.5 and 118.2 ppm. Peaks at (122.3, 6.7) and (117.5, 6.7) ppm have degenerate proton chemical shifts; therefore, the assumption was implied above that carbon atoms f/g resonate at higher field than d/e, since the former are second neighbors to the hydroxyl group. The observation that carbon d (f) does not correlate with proton e (g) indicates that ring flipping is much slower than in ibuprofen.

Hydrogen bonds play a significant role in polymorphism of acetaminophen.[22] The hydrogen-bond length of 1.79 ± 0.05 Å predicted from the chemical shift of 9.5 ppm by using Equation (1) agrees very well with a single-crystal X-ray diffraction structure, in which 1.77 Å was measured between the hydroxyl proton and the carbonyl oxygen atom of a neighboring molecule (Figure 3).[22] In a neutron diffraction structure this hydrogen bond length was 1.693 Å,[23] but sample differences (different unit-cell parameters and space group from the X-ray study) may also be responsible, in addition to the difference in accuracy of these techniques. For comparison, in the orthorhombic form the hydrogen-bond length is 1.84 Å (Cambridge Structural Database code HXACAN08).[22] Other intermolecular contacts can be revealed by further signal accumulation (Figure 2b). Proton a also correlates to carbon atoms c and h (Figure 2b), for which distances of 3.2 and 3.4 Å, respectively, are found in the structure (Figure 3). In addition, a three-bond intramolecular correlation is observed at (25.6, 9.1) ppm between carbon atom a and the amide proton (2.5 Å apart).

Notably, with fast MAS the majority of correlations arise from one-bond dipolar transfers, sharing the selectivity offered by the J-coupling mediated MAS-J-HMQC spectra with a short evolution period.[6,7] Fast MAS dramatically reduces the effective ^1H–^1H dipolar couplings and separates CH_n groups from other protons during cross polarization (without the need for homonuclear decoupling).[24] Meanwhile, quaternary carbon atoms correlate to protons two and three bonds away in the same spectrum. This feature has been essential in assigning aromatic resonances. In contrast, a separate MAS-J-HMQC experiment is necessary to establish multiple-bond correlations by using a very long evolution period, which leads to significant signal loss.[6,7] Longer-range correlations are very weak in fast-MAS HETCOR and can only be observed with additional signal accumulation. When long-range contacts are sought, for instance, for the determination of atomic-resolution structure,[25] they can be readily reestablished by inserting a ^1H–^1H dipolar recoupling element in the pulse sequence.[12]

As an alternative to fast-MAS HETCOR, proton double-quantum homonuclear correlation spectroscopy has recently been used to distinguish anhydrous and hydrous forms of a certain drug ingredient.[26] However, the CRAMPS technique (combined rotation and multiple-pulse spectroscopy) used there to narrow the proton linewidths also compromises signal-to-noise ratio as a result of the large receiver bandwidth and low probe quality factor. Moreover, CRAMPS experiments are difficult to calibrate and often show substantial variations in quality among samples. For these reasons, CRAMPS-based proton-detected HETCOR spectra of natural-abundance samples have not been demonstrated, to the best of our knowledge. Meanwhile, the proton homonuclear spectra correlating abundant protons (e.g., 2 h on 30-mg samples, recycle delay 2 s)[26] are comparable in sensitivity to the HETCOR spectra correlating protons with rare heteronuclei (^{13}C and ^{15}N), which have the benefit of very large chemical shift ranges. We expect the fast-MAS HETCOR experiments to have adequate resolution for molecules up to 500 Da, for which experiments can be conducted in 3 h given a favorable proton T_1. For even larger molecules (e.g., 1 kDa), we envision 3D experiments such as ^{13}C-^{1}H-^{1}H to provide additional resolution; such an experiment could be completed in a day, and the indirect proton dimension could be further improved in resolution by application of homonuclear decoupling, while the direct proton dimension would rely on fast MAS alone for high sensitivity.

Experimental Section

Sample preparation: A 310-mg ibuprofen tablet (Major Pharmaceuticals, Livonia, Michigan) containing 200 mg of API was crushed, and 6 mg was packed in a 1.6-mm NMR sample rotor. A 350-mg acetaminophen tablet (Ivax Pharmaceuticals Inc., Miami, Florida) containing 325 mg of API was crushed, and 6 mg was packed.

The solid-state NMR experiments were performed with 40-kHz MAS rate on a 750-MHz Varian INOVA spectrometer (Varian, Inc., Palo Alto, CA) with a FastMAS ^{1}H-^{13}C-^{15}N probe (Varian, Inc., Fort Collins, CO). Sample temperature was regulated at 11 °C; details of variable-temperature setup and calibration have been reported earlier.[12] The heteronuclear correlation pulse sequence employed the MISSISSIPPI technique to filter out background proton signals.[12] Contact times of 1.6 and 0.5 ms were used for the ^{1}H to ^{13}C and ^{13}C to ^{1}H cross-polarization steps, respectively. Other experimental details are listed in the figure captions. Chemical shifts were referenced indirectly to sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) by setting the downfield ^{13}C resonance of adamantane to 40.48 ppm.[27] Note that the ^{13}C chemical shifts on the DSS scale are 2.00 ppm larger than on the tetramethylsilane (TMS) scale, while the ^{1}H shifts are identical on both scales.[27]

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