Studies of minute quantities of natural abundance molecules using 2D heteronuclear correlation spectroscopy under 100 kHz MAS

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ABSTRACT

Two-dimensional $^1\text{H}\{^{13}\text{C}\}$ heteronuclear correlation solid-state NMR spectra of naturally abundant solid materials are presented, acquired using the 0.75-mm magic angle spinning (MAS) probe at spinning rates up to 100 kHz. In spite of the miniscule sample volume (290 nL), high-quality HSQC-type spectra of bulk samples as well as surface-bound molecules can be obtained within hours of experimental time. The experiments are compared with those carried out at 40 kHz MAS using a 1.6-mm probe, which offered higher overall sensitivity due to a larger rotor volume. The benefits of ultrafast MAS in such experiments include superior resolution in ^1H dimension without resorting to ^1H - ^1H homonuclear RF decoupling, easy optimization, and applicability to mass-limited samples. The HMQC spectra of surface-bound species can be also acquired under 100 kHz MAS, although the dephasing of transverse magnetization has significant effect on the efficiency transfer under MAS alone.

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INTRODUCTION

The availability of magic angle spinning (MAS) at ever increasing rates [1-4] has stimulated remarkable progress in the development of solid-state (SS)NMR methods for the studies of a wide range of materials, especially those comprising networks of strongly coupled ^1H nuclei. During the last decade, probeheads capable of MAS at 40-60 kHz have become increasingly common in many laboratories, owing their attractiveness to the superb RF capabilities and the ability to average, completely or partially, the anisotropic line-broadening interactions in many solid materials of interest in chemistry, materials science and biology. Multiple studies have highlighted the key advantages of such probeheads, which include excellent sensitivity per spin [5, 6], the ability to generate very strong RF magnetic fields, high cross-polarization (CP) efficiency [7, 8], and the possibility of using low-power RF in schemes requiring prolonged irradiation, e.g., during heteronuclear decoupling or polarization transfer [9-14]. The advent of fast MAS also spearheaded recent progress in the studies of paramagnetic systems, where suppression of large anisotropic paramagnetic broadening and improved decoupling capabilities led to large enhancements of both sensitivity and resolution [15, 16]. In addition, fast MAS serves to reduce the homonuclear dipolar couplings between high- γ nuclei, and is compatible with a number of RF pulse sequences designed for homonuclear ^1H decoupling [17-20]. The various combinations of these attributes led to the development of improved pulse sequences for multidimensional correlation studies. Notable examples include the multi-dimensional indirectly detected (^1H -detected or $^1\text{H}\{\text{X}\}$) heteronuclear correlation (HETCOR) experiments performed using through-space or through-bond polarization transfers, which offer greatly improved sensitivities compared the previously used X-detected schemes [21-24].

Ultrafast MAS, at rates approaching or even exceeding 100 kHz, stands to further expand these capabilities. Recent report has shown that the combination of MAS at 90 kHz and amplitude-modulated

low-power (12 kHz) heteronuclear ^1H decoupling produced ^{13}C coherence life time (T_2^*) for CH_2 groups in glycine comparable to that requiring 400 kHz ^1H decoupling under slow MAS [4]. Under such conditions, the residual line width of heteronuclei is likely to be governed by other factors, for instance magnetic susceptibility, dynamic effects and local disorder. Since MAS at 100 kHz exceeds the typical ^1H - ^1H dipolar couplings by a factor of ~ 2 , CRAMPS-quality ^1H resolution can be reached or approached in many solids without the use of RF decoupling [20], further stimulating the advancement and exploitation of multidimensional correlation methods [25-27]. For example, a homonuclear 2D ^1H - ^1H double quantum (DQ)MAS correlation spectrum acquired under MAS at 100 kHz yielded correlated distributions of chemical shifts with unforeseen resolution, thereby unraveling the structure of a complex host-guest system [25]. The enhanced ^1H resolution and longer T_2^* relaxation times favor the use of heteronuclear correlation methods hitherto limited mainly to solution NMR, including through-bond heteronuclear multiple quantum correlation (HMQC) schemes [26]. Most recently, the benefits of MAS at rates approaching 100 kHz were demonstrated in the study of a sub-milligram quantity of protein, where ^1H -detected 2D, 3D and 4D experiments utilizing heteronuclear single quantum correlation (HSQC) schemes were used to obtain a de novo structure of isotopically labeled ubiquitin [28].

Here, we demonstrate that despite the miniature rotor size, ultrafast MAS technology can serve to measure 2D $^1\text{H}\{^{13}\text{C}\}$ HSQC-type spectra of natural abundance materials, even those containing only the surface-bound functionalities. We show that the measurements are easy to optimize, remarkably sensitive, and compare well with those carried out using much larger rotors capable of MAS at 40 kHz. In favourable cases, the HMQC-type schemes can be used, as well.

EXPERIMENTAL

Samples. The well-studied sample of solvent-free, naturally abundant tripeptide (N-*formyl*-L-methionyl-L-leucyl-L-phenylalanine, referred to as f-MLF-OH) was chosen because it comprises CH, CH₂, CH₃ and quaternary carbon sites, which exhibit a full range of ¹³C-¹H couplings, from motionally narrowed to those typical of a fully rigid crystal lattice. The sample was purchased from Sigma-Aldrich.

As-MP-MSN, MP-MSN and phenetyl-HMDS-MSN. The organically functionalized mesoporous silica nanoparticles (MSNs) of MCM-41-type were synthesized as reported before [29]. In brief, a mixture of cetyltrimethylammonium bromide (CTAB, CH₃(CH₂)₁₅N(CH₃)₃Br) (1.0 g, 2.7 mmol), sodium hydroxide (3.5 mL, 2.0 M), and water (480 mL) was heated to 80°C in flask with constant stirring. After one hour, tetraethylorthosilicate (TEOS) (5.0 mL, 23 mmol) was added dropwise, which was followed by the addition of substituted trimethoxysilanes (3-mercaptopropyl trimethoxysilane, 1.0 mL, 5.1 mmol for MP-MSN, and phenetyl trimethoxysilane, 0.45 mL, 2.1 mmol for phenetyl-MSN). The mixtures were vigorously stirred at 80°C for 2 hours and filtered. The resulting white solids were washed with copious amount of water and methanol and dried under vacuum overnight, yielding the as-synthesized materials as-MP-MSN and as-phenetyl-MSN.

To remove the surfactant template, 1.0 g of as-MP-MSN or as-phenetyl-MSN was suspended in methanol (100 mL), concentrated HCl was added (1.0 mL) and the mixture was refluxed for 6 h. The solid was then recovered by filtration and washed with copious amounts of water and methanol, and then dried under vacuum overnight. The extracted materials are referred to as MP-MSN and phenetyl-MSN.

Textural properties of these materials were measured by nitrogen sorption isotherms at -196 °C in a Micromeritics Tristar analyzer. The surface areas were calculated by the Brunauer-Emmett-Teller (BET) method, and the pore size distribution was calculated by the Barrett-Joyner-Halenda (BJH) method. Pretreatment of samples for surface area measurement was done by flowing N₂ for 6 h at 100 °C. MP-

MSN had a surface area of 980 m²/g, 2.2 nm pore width and a pore volume of 0.54 cm³/g; the corresponding values for phenethyl-MSN were 1149 m²/g, 2.45 nm and 1.07 cm³/g.

The loading of organic groups in MP-MSN was determined with the Perkin Elmer 2100 Series II CHN/S Elemental Analyzer, using acetanilide as a standard, and combustion and reduction at 925 °C and 640 °C. The amount of mercaptopropyl groups in MP-MSN was found to be 2.45 mmol/g.

Phenethyl-MSN (0.4 g) was further reacted with hexamethyldisilazane (HMDS) (2.0 mL, 9.6 mmol) by refluxing in hexanes (100 mL) for 24 h. The final product (phenethyl-HMDS-MSN) was recovered by filtration, washed with copious amounts of hexanes, and dried under vacuum overnight.

Solid-state NMR measurements. All measurements were performed at 14.1 T, on a JEOL ECA600 spectrometer (0.75-mm probe, operated at a MAS rate $\nu_R = 90$ -100 kHz) and a 600 MHz Varian NMR System spectrometer (1.6-mm probe, $\nu_R = 40$ -42 kHz). Basic sensitivity tests were performed using 1D MAS spectra measured under direct polarization (DPMAS). The 2D pulse sequences followed the HSQC and HMQC schemes used in solution NMR [30, 31], with various modifications specific to solids (Fig. 1) [20-23]. In both experiments, the ¹³C magnetization evolving during t_1 under the isotropic chemical shift is transferred to ¹H nuclei and subsequently detected during t_2 . As a reference, we also carried out a ¹³C-detected experiment using a 1.6-mm probe, where PMLG homonuclear ¹H-¹H decoupling was applied during t_1 to maximize the ¹H resolution (pulse sequence not shown) [17, 18, 32].

As detailed below, the sequences used double-quantum (DQ) or zero-quantum (ZQ) Hartmann-Hahn CP conditions [33], and CW, SPINAL-64 or WALTZ heteronuclear decoupling [34, 35], as appropriate, using low RF magnetic fields of $\sim \nu_R/4$ or less. The uncorrelated ¹H magnetization has been purged during the HORROR recoupling period, as reported earlier [36, 37]. The HORROR condition was established by minimizing the ¹H NMR signal following a spin-locking pulse, first by setting the spin-lock RF magnetic field strength at $\sim \nu_R/2$, then by optimizing the duration of the spin-locking pulse τ_{RR} .

States-TPPI quadrature detection was used by switching appropriately the phase of the first $\pi/2$ pulse in the ^{13}C channel.

The experimental parameters are given in the figure captions, using the following symbols: ν_R indicates the sample rotation rate, B_{RF}^X the magnitude of RF magnetic field at the frequency of X nuclei, τ_{CP1} the CP time for the first ($^1\text{H} \rightarrow ^{13}\text{C}$) polarization transfer, τ_{CP2} the CP time for the second ($^{13}\text{C} \rightarrow ^1\text{H}$) polarization transfer, τ_{RR} is the rotary resonance recoupling period, τ_{RD} the recycle delay, Δt_1 the time increment during t_1 evolution, NS the number of scans, and AT the total acquisition time. All spectra were recorded using purge gas, with the rotor temperature increased above the ambient by $\sim 15^\circ\text{C}$ at $\nu_R = 40\text{ kHz}$ and by $\sim 30^\circ\text{C}$ at $\nu_R = 100\text{ kHz}$. The proton and carbon chemical shifts δ_{H} and δ_{C} are referenced to TMS at 0 ppm.

Fig. 1. (A) CP-based HSQC-type and (B) HMQC pulse sequences used in this study. In the HSQC measurements, ^1H magnetization is transferred to ^{13}C spins followed by ^{13}C free precession under low-power CW or SPINAL-64 ^1H decoupling during the t_1 period. The residual ^1H magnetization is suppressed by phase-altered HORROR irradiation during τ_{RR} . The evolution of ^{13}C magnetization is observed indirectly after the second CP transfer, via ^1H precession under WALTZ or SPINAL-64 ^{13}C decoupling.

RESULTS AND DISCUSSION

Optimization of 2D measurements

0.75-mm MAS probe. The experimental conditions were optimized by observing NMR spectra of $^{13}\text{C}_3$, ^{15}N -L-alanine. It has been our experience that CP transfers under DQ Hartmann-Hahn matching condition

$$\eta_{\text{RF}}^{\text{H}} + \eta_{\text{RF}}^{\text{C}} = n\eta_{\text{R}} \quad (1)$$

yielded slightly better signal intensities (by a few percent in our tests) than the traditional ZQCP format

$$\eta_{\text{RF}}^{\text{H}} - \eta_{\text{RF}}^{\text{C}} = \pm n\eta_{\text{R}} \quad (2)$$

Additionally, the use of weaker RF magnetic field lessens the burden of high-power on the NMR probe and lowers the risk of overheating the sample. Thus, the DQCP matching condition has been used in all our experiments utilizing the 0.75-mm probe.

The DQCP conditions for both CP1 and CP2 proved easy to optimize using the following procedure.

First, we roughly maximized the $^1\text{H} \rightarrow ^{13}\text{C}$ CP transfer with $\tau_{\text{CP}} = 2$ ms, by adjusting $\eta_{\text{RF}}^{\text{H}}$ and $\eta_{\text{RF}}^{\text{C}}$ (including ramp in the ^{13}C channel). As a general rule, we used $\eta_{\text{RF}}^{\text{C}} > \eta_{\text{RF}}^{\text{H}}$ to cover the broader ^{13}C chemical shift range. Specifically, two DQCP conditions were targeted, referred to as DQH30 (where $\eta_{\text{RF}}^{\text{H}} = 30$ kHz and $\eta_{\text{RF}}^{\text{C}} = 70$ kHz) and DQH10 ($\eta_{\text{RF}}^{\text{H}} = 10$ kHz and $\eta_{\text{RF}}^{\text{C}} = 90$ kHz). Subsequently, the CP1 and CP2 conditions were fine-tuned, including the sense of ramp, by monitoring ^1H signal intensities using the sequence shown in Fig. 1A with $t_1 = 0$. We used DQH30 condition with $\tau_{\text{CP1}} = 2$ ms to attain optimal efficiency and uniformity of CP1 ($^1\text{H} \rightarrow ^{13}\text{C}$) polarization transfer. On the other hand, the DQH10 condition with short τ_{CP2} value of 0.2 to 0.4 ms is preferred for CP2 to enhance the selectivity of magnetization transfer between bonded ^1H - ^{13}C pairs. Under ultrafast MAS the heteronuclear dipolar interactions between bonded ^1H - ^{13}C pairs dominate the spin dynamics during ^{13}C -

^1H CP, leading to fast and efficient polarization build-up (with the first maximum at $\sim 70 \mu\text{s}$ [38]) and strong truncation of long-range transfers. We finally note that in the presence of ^{13}C and ^1H resonance offsets, $\text{Dn}_{\text{RF}}^{\text{C}}$ and $\text{Dn}_{\text{RF}}^{\text{H}}$, the DQCP Hartmann-Hahn with $n = 1$ can be written as

$$\sqrt{(n_{\text{RF}}^{\text{H}})^2 + (\text{Dn}_0^{\text{H}})^2} + \sqrt{(n_{\text{RF}}^{\text{C}})^2 + (\text{Dn}_0^{\text{C}})^2} = n_{\text{R}}. \quad (3)$$

Although we did not carry out systematic measurements of CP efficiencies as a function of $\text{Dn}_{\text{RF}}^{\text{C}}$, it is advisable to place the ^{13}C carrier frequency near the center of the spectrum, in addition to using the ramped CP schemes, to maximize the uniformity of polarization transfers.

1.6-mm MAS probe. The 2D HETCOR experiments performed at $\nu_{\text{R}} = 40$ or 41.667 kHz used tangent CP1 and CP2 transfers, with n_{RF}^{H} and n_{RF}^{C} of around 60 kHz and 100 kHz , respectively.

Results

Sensitivity of 1D DPMAS. To estimate of the relative sensitivity in experiments carried out with 1.6-mm and 0.75-mm MAS probes, we measured 1D DPMAS spectra of hexamethylbenzene. The spectra were acquired using fully loaded rotors, under equivalent conditions, including the MAS rate (40 kHz). The measurements (not shown), yielded the S/N ratios that differed by a factor of ~ 12 in favor of the larger rotor.

This outcome is not unexpected: the overall loss of signal due to smaller samples size is only partly offset by higher sensitivity per unit volume. At a given magnetic field and temperature, the signal to noise (S/N) ratio of a small-size coil is proportional to [39]

$$S/N \propto \frac{B_1 V}{\sqrt{R_{\text{coil}}}}, \quad (4)$$

where V is the sample volume, R_{coil} denotes the coil resistance due to conducting losses as well as skin and proximity effects, and B_1 is the magnitude of the transverse RF magnetic field induced in the coil by a unit current, which in a single-layer solenoid coil is determined by the coil length l , coil diameter d_{coil} ,

and the number of turns m [40],

$$B_1 = \frac{m}{d_{\text{coil}} \sqrt{1 + (l/d_{\text{coil}})^2}} . \quad (5)$$

Despite their small size, both coils used in our experiments operate in the skin depth regime (the skin depth of copper is a few μm at the frequencies of our interest), where

$$R_{\text{coil}} = \frac{m^2 d_{\text{coil}}}{l} . \quad (6)$$

From (4), (5) and (6) it follows that for coils with the same value of l/d_{coil} the dependency of S/N ratio per unit volume should scale as $(d_{\text{coil}})^{-1}$.

In the abovementioned DPMAS measurements of hexamethylbenzene, the spectra were acquired with $d_{\text{coil}} \cong 1.2$ mm and the active sample volume of $V = 290$ nL (0.75-mm probe), and $d_{\text{coil}} \cong 2.2$ mm and $V \cong 6$ μL (1.6-mm probe). Disregarding possible differences in the receiver noise levels in JEOL and Varian spectrometers, RF magnetic field inhomogeneities, probe circuit design, sample packing, sample heating, etc., and considering only the product of sample volume and relative sensitivity, should give a factor of $(6000/290)/(2.2/1.2) \cong 11$ in favor of a larger rotor, which indeed agrees very well with the result of our measurement. Thus, in standard 1D experiments the use of ultrafast MAS imposes a severe penalty on sensitivity. However, this technology will be applicable in experiments requiring high ^1H resolution (especially ^1H -detected HETCOR), very high RF fields, or for samples with limited mass.

2D HETCOR spectra of MLF. In Figs. 2A-C, we compare 2D ^1H - ^{13}C HETCOR spectra of f-MLF-OH tripeptide taken under the following conditions: (A) $^1\text{H}\{^{13}\text{C}\}$, $\nu_{\text{R}} = 100$ kHz, $\tau_{\text{CP1}} = 2$ ms, and $\tau_{\text{CP2}} = 0.4$ ms (referred to as DQH30_2ms_DQH10_0.4ms); (B) $^1\text{H}\{^{13}\text{C}\}$, $\nu_{\text{R}} = 41,667$ kHz, $\tau_{\text{CP1}} = 2$ ms, and $\tau_{\text{CP2}} = 0.4$ ms; and (C) $^{13}\text{C}\{^1\text{H}\}$ (note that this is a ^{13}C -detected spectrum), $\nu_{\text{R}} = 41,667$ kHz, and $\tau_{\text{CP}} = 0.4$ ms. All spectra were optimized as explained above and acquired under similar experimental

conditions in 5 h (A) and 10 h (B, C). A complete list of acquisition parameters is given in the figure caption. The peaks assignments are shown in Table 1 and Scheme 1, based on the earlier studies by Griffin et al. [41, 42]. We observe that there are some notable differences between relative peak intensities in the spectra in Fig. 2 due to various efficiencies of $^1\text{H} \rightarrow ^{13}\text{C}$ and $^{13}\text{C} \rightarrow ^1\text{H}$ CP transfers in our experiments. They are most significant for the carbonyl groups, which were clearly detected under 100 kHz MAS using $\tau_{\text{CP}2} = 2$ ms (DQH30_2ms_DQH10_2ms, spectrum not shown), and phenyl rings, which are known to undergo 180° flips at a rate of $\sim 10^5 \text{ s}^{-1}$ [42], and are essentially absent in the spectra acquired at 100 kHz MAS.

Scheme 1. Molecular structure of f-MFL-OH.

Notwithstanding these differences, an estimate of the S/N ratios in the spectra of Figs. 2A and 2B indicates that the relative sensitivity adjusted for the number of scans is on average only about 1-2 times better in spectrum (B), which represents a considerable shift in favor of the 0.75-mm probe compared to 1D DPMAS spectra. This notable result can be mostly attributed to improved resolution in the ^1H dimension at 100 kHz MAS (here, by a factor of approximately 2) and, to a lesser extent, in the ^{13}C dimension (here, on average by 20%).

Another factor affecting the observed sensitivities, albeit difficult to quantify precisely, is that the electronic circuit of the 0.75-mm probe was optimized for ^1H detection. We can only note that the JEOL 0.75-mm probe delivered $\eta_{\text{RF}}^{\text{H}} = 490$ kHz at 60 W power, and $\eta_{\text{RF}}^{\text{C}} = 221$ kHz at 100 W power. For the Varian 1.6-mm probe, the corresponding values are $\eta_{\text{RF}}^{\text{H}} = 235$ kHz and $\eta_{\text{RF}}^{\text{C}} = 177$ kHz.

We also acquired a ^{13}C -detected spectrum with PMLG homonuclear ^1H - ^1H decoupling during the ^1H evolution period t_1 (Fig. 2C). The resulting spectrum exhibits state-of-the-art resolution, which slightly exceeds one delivered by MAS at 100 kHz. However, the $^{13}\text{C}\{^1\text{H}\}$ HETCOR experiment lacks the sensitivity benefit of ^1H -detection, and in effect yields similar sensitivity per unit time than the $^1\text{H}\{^{13}\text{C}\}$ measurement in Fig. 2A, which further underscores the merit of ultrafast MAS. Another important fact to note is that in accordance with our earlier comments, the only correlations observed in Fig. 2A are those between directly bonded ^1H - ^{13}C pairs. This is in contrast to a spectrum reported in our earlier study, where at $\nu_R = 41.667$ kHz and $\tau_{\text{CP2}} = 0.3$ ms multiple long-range correlations were observed [32]. Most likely, ultrafast MAS significantly reduced ^1H - ^1H couplings and accordingly the long-range ^1H - ^{13}C correlations during the CP2 period, producing a spectrum similar to J -based schemes. Further study will help to understand the behavior of long-range ^1H - ^{13}C correlations under ultrafast MAS.

Fig. 2. 2D ^1H - ^{13}C HSQC-type spectra of f-MLF-OH taken at 14.1 T under the following conditions: (A) $^1\text{H}\{^{13}\text{C}\}$, $\nu_R = 100$ kHz, $\tau_{\text{CP1}} = 2$ ms, $\tau_{\text{CP2}} = 0.4$ ms, (DQH30_2ms_DQH10_0.4ms), $\tau_{\text{RR}} = 10$ ms, $\eta_{\text{RF}}^{\text{H}} = 10$ kHz during CW ^1H decoupling, $\eta_{\text{RF}}^{\text{H}} = 50$ kHz during τ_{RR} , and $\eta_{\text{RF}}^{\text{C}} = 10$ kHz during WALTZ ^{13}C decoupling; (B) $^1\text{H}\{^{13}\text{C}\}$, $\nu_R = 41,667$ kHz, $\tau_{\text{CP1}} = 2$ ms, $\tau_{\text{CP2}} = 0.4$ ms, $\tau_{\text{RR}} = 40$ ms, $\eta_{\text{RF}}^{\text{H}} \approx 60$ kHz during tangent τ_{CP1} and τ_{CP2} , $\eta_{\text{RF}}^{\text{H}} = 10$ kHz during SPINAL-64 ^1H decoupling, $\eta_{\text{RF}}^{\text{H}} = 20.85$ kHz during τ_{RR} , $\eta_{\text{RF}}^{\text{C}} = 102$ kHz during τ_{CP1} and τ_{CP2} , and $\eta_{\text{RF}}^{\text{C}} = 10$ kHz during SPINAL-64 ^{13}C decoupling; and (C) $^{13}\text{C}\{^1\text{H}\}$, $\nu_R = 41,667$ kHz, $\tau_{\text{CP}} = 0.4$ ms, $\eta_{\text{RF}}^{\text{H}} \approx 64$ kHz during tangent τ_{CP} , $\eta_{\text{RF}}^{\text{H}} = 10$ kHz during SPINAL-64 ^1H decoupling, $\eta_{\text{RF}}^{\text{C}} = 106$ kHz during τ_{CP} , and $\eta_{\text{RF}}^{\text{C}} = 10$ kHz during SPINAL-64 ^{13}C

decoupling. In addition PMLG decoupling with $\nu_{\text{RF}}^{\text{H}} \approx 160$ kHz was used during t_1 in (C). All spectra were acquired in 256 rows with $\Delta t_1 = 24$ μs and $\tau_{\text{RD}} = 2.25$ s. In (A), the number of scans per raw was $\text{NS} = 16$, resulting in $\text{AT} \approx 5$ h, while in (B) and (C) the acquisition time was increased twofold to $\text{NS} = 32$ and $\text{AT} \approx 10$ h. The spectra were processed using the same parameters and are accompanied by equivalent ^1H and ^{13}C skyline projections.

Table 1 The spectral assignments in f-MLF-OH.

	δ_{C} [ppm]		δ_{C} [ppm]		δ_{C} [ppm]
M^{α}	52.0	L^{α}	56.8	F^{α}	54.3
M^{β}	37.5	L^{β}	40.6	F^{β}	36.9
M^{γ}	28.7	L^{γ}	25.0	F^{γ}	$\sim 130^{\text{a}}$
M^{δ}	14.0	$\text{L}^{\delta 1}$	24.6	F^{δ}	$\sim 130^{\text{a}}$
$\text{M}^{\text{carbonyl}}$	172.1	$\text{L}^{\delta 1}$	19.6	F^{ϵ}	$\sim 130^{\text{a}}$
M^{formyl}	165.2	$\text{L}^{\text{carbonyl}}$	174.8	F^{ζ}	127.6
				$\text{F}^{\text{carboxyl}}$	173.2

^a unresolved due to ring dynamics

2D HETCOR spectra of MSNs. The $^1\text{H}\{^{13}\text{C}\}$ HSQC spectra of MP-MSN acquired using 0.75-mm and 1.6-mm probes (here at $\nu_{\text{R}} = 90$ kHz and $= 40$ kHz, respectively) are shown in Fig. 3A and 3B. Based on the ^{13}C spectra published in our earlier study [43], the main observed cross-peaks are ascribed to Si-**CH**₂-CH₂-CH₂-SH (**a**), Si-CH₂-**CH**₂-CH₂-SH (**b**), and Si-CH₂-CH₂-**CH**₂-SH (**c**) in MP groups. Here the resolution is not affected by MAS, and the sensitivity per scan is estimated at $\sim 1/5$ of that of the ‘larger’ 1.6-mm probe. According to the elemental analysis, the MP-MSN material contained 2.45 mmol/g of natural abundance MP functional groups bound to the silica surface. Thus, the spectrum in Fig. 3A was derived from just about 10^{16} pairs of ^{13}C - ^1H nuclei decorating roughly 0.25 m^2 of silica surface. Remarkably, a similar 2D HETCOR spectrum could be detected from just 25 cm^2 of silica surface by using ^{13}C -enrichment.

Fig. 3. 2D ^1H - ^{13}C HSQC-type spectra of MP-MSN taken at 14.1 T under the following conditions: (A) $^1\text{H}\{^{13}\text{C}\}$, $\nu_{\text{R}} = 90$ kHz; $\tau_{\text{CP1}} = \tau_{\text{CP2}} = 2$ ms (DQH30_2ms_DQH30_2ms), $\tau_{\text{RR}} = 10$ ms, $\eta_{\text{RF}}^{\text{H}} = 10$ kHz during CW ^1H decoupling, $\eta_{\text{RF}}^{\text{H}} = 45$ kHz during τ_{RR} , $\eta_{\text{RF}}^{\text{C}} = 10$ kHz during WALTZ ^{13}C decoupling; (B) $^1\text{H}\{^{13}\text{C}\}$, $\nu_{\text{R}} = 40$ kHz; $\tau_{\text{CP1}} = \tau_{\text{CP2}} = 2$ ms, $\tau_{\text{RR}} = 40$ ms, $\eta_{\text{RF}}^{\text{H}} = 60$ kHz during tangent τ_{CP1} and τ_{CP2} , $\eta_{\text{RF}}^{\text{H}} = 10$ kHz during SPINAL-64 ^1H decoupling, $\eta_{\text{RF}}^{\text{H}} = 20$ kHz during τ_{RR} , $\eta_{\text{RF}}^{\text{C}} = 100$ kHz during τ_{CP1} and τ_{CP2} , and $\eta_{\text{RF}}^{\text{C}} = 10$ during SPINAL-64 ^{13}C decoupling. Spectrum (A) was acquired in 64 rows, with $\Delta t_1 = 26.5$ μs , 384 scans per row, $\tau_{\text{RD}} = 1$ s, and AT = 13.7 h. Spectrum (B) was acquired in 64 rows, with $\Delta t_1 = 25$ μs , 96 scans per row, $\tau_{\text{RD}} = 1$ s, and AT = 3.8 h. The spectra were processed using the same parameters and are accompanied by equivalent ^1H and ^{13}C skyline projections.

Fig. 4. 2D $^1\text{H}\{^{13}\text{C}\}$ HMQC-type spectra of (A) as-MP-MSN and (B) phenethyl-HMDS-MSN taken at

14.1 T under the following conditions: (A) $^1\text{H}\{^{13}\text{C}\}$, $\nu_{\text{R}} = 100$ kHz, and $\tau = 3$ ms; (B) $^1\text{H}\{^{13}\text{C}\}$, $\nu_{\text{R}} = 100$ kHz (97 kHz for inset), and $\tau = 3$ ms. Spectrum (A) was acquired in 256 rows, with $\Delta t_1 = 4$ μs , 8 scans per row, $\tau_{\text{RD}} = 1.35$ s, and AT = 1.5 h. Spectrum (B) was acquired in 64 rows, with $\Delta t_1 = 26.48$ μs , 2 scans (256 scans for the inset) per row, $\tau_{\text{RD}} = 2$ s (1.7 s for the inset), and AT = 8.5 min (15.5 h for the inset). The spectra were processed using the same parameters and are accompanied by equivalent ^1H and ^{13}C skyline projections.

In the solid-state HMQC experiments the ^{13}C - ^1H transfers can be achieved via J coupling (J -HMQC) or dipolar coupling (D -HMQC). Here, we carried out the J -HMQC measurements using the pulse sequence shown in Fig. 1B. In addition to providing through-bond filtering, the advantages of the J -HMQC over D -HMQC include robustness and immunity to the t_1 -noise problems induced in the indirect dimension D -HMQC spectra by the fluctuations of MAS frequency [26]. The main shortcoming of the J -HMQC scheme is susceptibility to T_2^{eff} relaxation effects during polarization transfers, each requiring several ms ($t \approx 1/2J$), and during t_1 evolution.

Both the benefits and shortcomings of the J -HMQC experiment are on display in the spectra of Fig. 4. The transfers involving ^1H nuclei with long T_{2H}^{eff} times proved to be very efficient. This is evident in the spectrum of as-MP-MSN (Fig. 4A), which features strong resonances representing most of the expected ^{13}C - ^1H correlations in CTAB. However, the correlations due to mercaptopropyl groups are notably absent, despite being easily detected in the HSQC spectrum of the same sample after the surfactant had been extracted (Fig. 3).

Similarly, Fig. 4B shows a 2D HMQC spectrum of surface-bound species of the phenethyl-HMDS-MSN sample, again containing merely $\sim 10^{16}$ of ^{13}C - ^1H pairs, which was measured in less than 9 min. The cross-peaks involving the aromatic group of phenethyl and methyl groups in HMDS, with T_{2H}^{eff} of 17 and 26 ms, respectively, were detected with very high efficiency, exceeding 50% of the intensity of the properly scaled ^1H DPMAS spectrum. On the other hand, even at $\nu_{\text{R}} = 100$ kHz the correlations involving sites with shorter T_{2H}^{eff} times were strongly suppressed, and could only be detected after a

much longer acquisition. Indeed, the methylene groups **a** and **b** in phenetyl groups, with T_{2H} values of 11 ms and 10 ms, respectively, can be recognized in the spectrum of the same sample acquired in 15 h (see the inset in Fig. 4B).

CONCLUSION

We demonstrated that high-quality 2D HETCOR $^1\text{H}\{^{13}\text{C}\}$ spectra of natural abundance samples can be conveniently obtained under MAS at 100 kHz. Despite the miniature volume of 0.75-mm MAS rotor, even surface-bound functionalities comprising just 10^{16} of ^{13}C - ^1H pairs can be studied. The HSQC-type experiments are easy to optimize and yield sensitivity in that comes surprisingly close to that achieved in much larger 1.6-mm rotors capable of MAS at 40 kHz. It should be noted, however, that decoherence of transverse ^1H magnetization of strongly coupled spins is generally too fast to allow for similar efficiency in HMQC-type measurements spectra. This shortcoming can be alleviated by using ^1H - ^1H homonuclear dipolar decoupling.

The ultrafast MAS technology offers high efficiency of heteronuclear polarization transfer, opportunities for spectral editing hitherto limited to solution NMR, and fundamentally new decoupling/recoupling capabilities. These effects institute the gradual convergence of solid-state and solution NMR and lend themselves to further experimental and theoretical investigation. Their applications will include a variety of energy-related materials, including complex hydrides, thermoelectric materials, biological solids and heterogeneous catalysts.

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