NMR spectroscopy

High-Resolution NMR Spectroscopy in Solids by Truly Magic-Angle Spinning**

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There is an ever increasing interest in obtaining high-resolution NMR spectra of $S = 1/2$ nuclei, such as ^{13}C, in solids. Solid-state NMR spectroscopy is important for material science, for (bio)organic chemistry, for protein structure determination,[1–3] and for the characterization of pharmaceutical products (e.g., crystalline polymorphism).[4] The combination of magic-angle spinning (MAS) with heteronuclear dipolar decoupling leads to line narrowing, and hence to an improvement of both resolution and sensitivity (peak-height-to-noise ratio). Herein, we show that the line width of ^{13}C resonances can be narrowed to 0.039 ppm (3.9 Hz for ^{13}C at 100.6 MHz or 9.4 T). Such a narrow resonance is observed for carbonyl carbon atoms of polycrystalline cholesteryl acetate if the magic angle ($\theta_m = \arccos 3^{-1/2} \approx 54.736°$) is adjusted very accurately, that is, within $|\Delta\theta| = |\theta - \theta_m| = 0.004°$, as commonly done for satellite-transition magic-angle spinning (STMAS) NMR spectroscopy of quadrupolar nuclei.[5,6] The lower limit of the line width (which is inversely proportional to the effective spin-echo decay time constant T_2') can be as little as 0.09 Hz for carbonyl carbon atoms in choresteryl acetate. We also demonstrate by ^{207}Pb NMR spectroscopy that temperature gradients across the sample (which lead to a distribution of isotropic chemical shifts) can provide an important contribution to the line width, in addition to imperfect decoupling,[7] structural disorder,[8] and magnetic susceptibility effects.[8–10]

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A common way to adjust the magic angle is to optimize the envelope of ^{79}Br rotational echoes in KBr. Herein we suggest a procedure that is more sensitive, and involves two steps: First, the angle can be adjusted to an accuracy of $|\Delta\theta| < 0.01°$ ($\Delta\theta$ = deviation from the magic angle) by minimizing the residual ^2H quadrupolar splitting in a rotor-synchronized MAS spectrum of a deuterated sample, such as $[D_6]\alpha$-oxalic acid dihydrate. The residual splitting (Figure 1) allows the angle θ to be determined if it is miss-set by more than $|\Delta\theta| = 0.01°$. A fine adjustment to an accuracy $|\Delta\theta| < 0.004°$ can be achieved by maximizing the height of the $ST_1 \rightarrow CT$ shifted-echo signal in a one-dimensional ^{87}Rb STMAS spectrum of $RbNO_3$.[6]

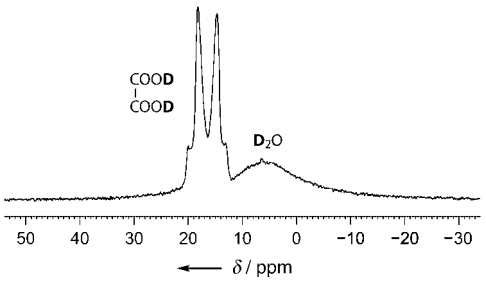

Figure 1. Rotor-synchronized ^2H MAS spectrum (spinning speed $\nu_R = 20$ kHz) of $[D_6]\alpha$-oxalic acid dihydrate. The broad hump to the right stems from motionally averaged water deuterons.[11] The residual quadrupolar powder pattern to the left, which stems from the carboxyl deuterons, allows the deviation from the magic angle to be estimate as $\Delta\theta = -0.036$.

Samples packed in 2.5 mm outer diameter ZrO_2 rotors were spun at 30 kHz (unless otherwise stated) in a standard Bruker triple resonance CPMAS probe in a 9.4 T widebore magnet of an Avance 400 spectrometer. The static field homogeneity was shimmed for ^{13}C line widths in adamantane $\Delta\nu = 3$ Hz (full width at half-height). Cross-polarization (CP) was used with a proton radio-frequency (RF) amplitude of 85 kHz. Two-pulse phase modulation (TPPM) proton decoupling was used during signal acquisition with 100 kHz RF amplitude, pulse-widths of 3.9 μs, and a phase difference between two successive pulses of 35°. Amino acids (Cambridge Isotope Laboratories), and cholesteryl acetate (Fluka) were used without further purification or recrystallization.

Figure 2a shows the ^{13}C CPMAS spectrum of cholesteryl acetate recorded at the magic angle. The line widths $\Delta\nu = 1/(\pi T_2^*)$ (full width at half-height) of the ^{13}C resonances range from 3.9 Hz for the carbonyl carbon to 12.6 Hz for some protonated carbons atoms (Table 1), and are even narrower than previously reported.[12] The width $\Delta\nu = 3.9$ Hz (only 0.9 Hz more than the line width of the plastic crystalline adamantane) is, to our knowledge, the narrowest ^{13}C resonance reported for polycrystalline solids. Intentional deviations from the magic angle $\Delta\theta = 0.134°$ and 0.234° (Figure 2b, c) lead to an increase in the line widths. Not all resonances are broadened to the same extent. The carbonyl signals enlarged in Figure 3 feature residual chemical shift anisotropy (CSA) powder line shapes that are scaled by $(3\cos^2\theta - 1)/2 = -0.00333$, $+0.00211$, and 0 for Figure 3a, b, and c, respec-

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Table 1: Twelve ^{13}C resonances in cholesteryl acetate.[a]

δ_{iso} [ppm]	170.90	141.47	121.78	73.49	52.71	41.57	37.48	34.27	32.84	28.48	21.32	13.89
$1/(\pi T_2^*)$ [Hz]	3.9	5.2	7.3	6.0	9.4	9.1	4.1	12.6	6.6	6.9	4.8	10.1
T_2' [ms]	3600	2300	130	160	150	60	420	70	150	90	370	280
$1/(\pi T_2')$ [Hz]	0.09	0.14	2.4	2.0	2.1	5.3	0.75	4.5	2.1	3.5	0.86	1.1

[a] Apparent line widths $\Delta\nu = 1/(\pi T_2^*)$, time constants T_2' of echo decays, and corresponding homogeneous (best possible) line widths $1/(\pi T_2')$. Partial assignments can be found in the work of De Paëpe et al.[12]

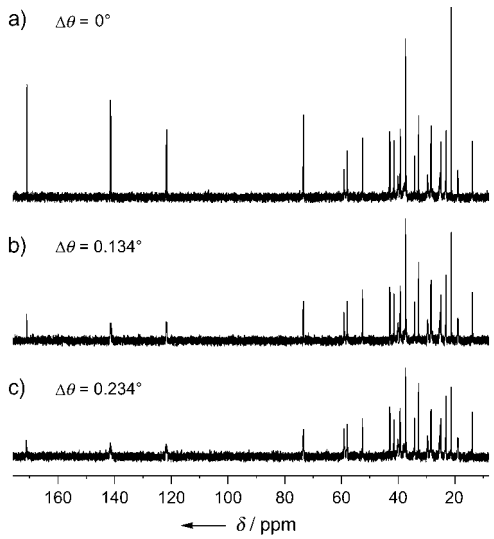

Figure 2. ^{13}C CPMAS spectra of cholesteryl acetate recorded at 9.4 T (100.6 MHz) as a function of the angle θ a) at the magic angle, that is, $\Delta\theta = 0$, b) and c) at deviations from the magic angle. The CP contact time was 1 ms, the recycle interval 4 s, the acquisition time 0.273 s for each of 1280 transients. No line broadening was applied.

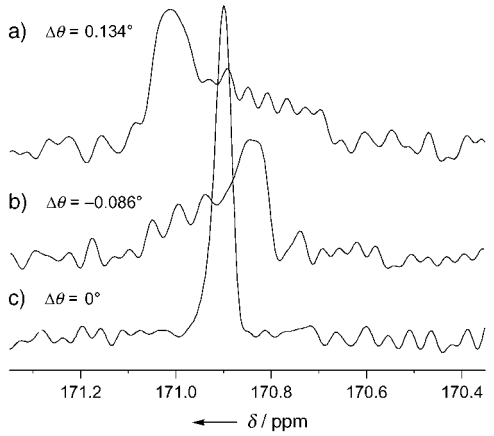

Figure 3. ^{13}C CPMAS resonances of the carbonyl carbon in cholesteryl acetate recorded as a function of the angle θ (spectrum c) at the magic angle, that is, $\Delta\theta = 0$. b) and a) at deviations from the magic angle, featuring scaled powder patterns characteristic of a symmetrical CSA tensor. With scaling factors $(3\cos^2\theta - 1)/2 = -0.00333$ (a) and $+0.00211$ (b) predicted from residual observed quadrupolar splittings in ^2H MAS spectra, powder patterns can be simulated that closely match the two spectra observed with $\Delta\theta \neq 0$ if the CSA principal components are assumed to be $\delta_{xx} = \delta_{yy} = 138.9$ ppm and $\delta_{zz} = 234.9$ ppm. The widths of the scaled powder patterns are directly proportional to the static magnetic field ($B_0 = 9.4$ T in this case).

tively, corresponding to $\Delta\theta = 0.134°$, $-0.086°$, and $0°$. It is remarkable that the residual scaled CSA effects are not masked by broadening owing to imperfect decoupling, structural disorder, temperature gradients, and magnetic susceptibility effects.

In comparison to cholesteryl acetate, amino acids often exhibit greater line widths, usually ascribed to magnetic susceptibility and structural disorder. The accurate adjustment of the magic angle also plays a significant role as demonstrated in ^{13}C CPMAS spectra of L-alanine (Figure 4).

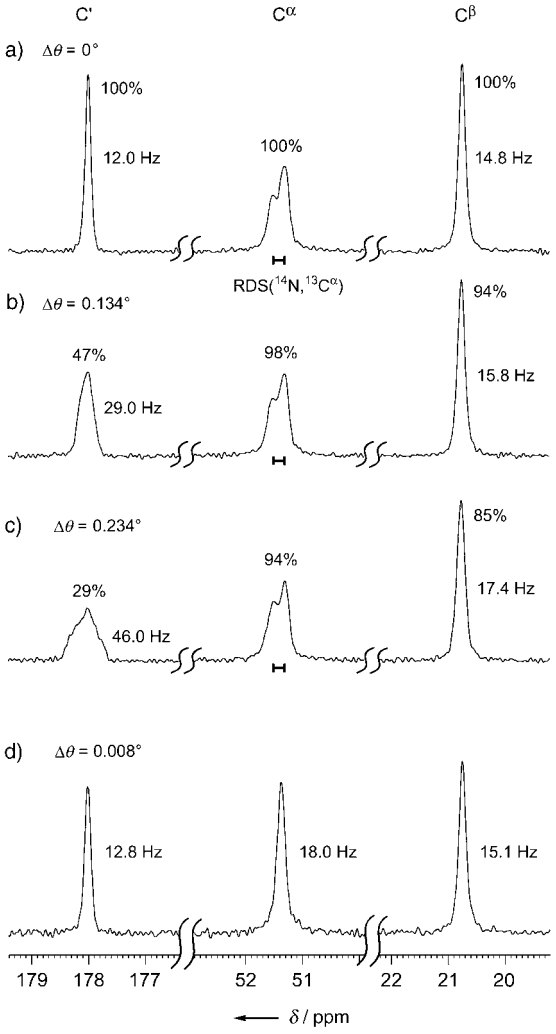

Figure 4. ^{13}C CPMAS spectra of a)–c) [^{14}N]L-alanine at different $\Delta\theta$ values and d) [^{15}N]L-alanine. The spectra result from averaging 640 transients with a recycle interval of 2 s. The CP contact time was 500 μs. The acquisition time was 0.136 s. No line broadening was applied.

For $\Delta\theta \neq 0$, the main contributions to the line widths again originate from residual CSA effects. The CSA tensors in L-alanine, determined independently by Ye et al.,[13] give $\delta_{CSA} = -70, -20, -12$ ppm and $\eta_{SA} = 0.79, 0.35$ and 1 for C′, C^{α} and C^{β}, respectively, using the definitions $\delta_{CSA} = \delta_{zz} - \delta_{iso}$, $\eta_{SA} = (\delta_{yy} - \delta_{xx})/\delta_{CSA}$, and re-ordering the principal components so that $|\delta_{zz} - \delta_{iso}| \geq |\delta_{xx} - \delta_{iso}| \geq |\delta_{yy} - \delta_{iso}|$. The line widths of the C′ and C^{β} resonances of L-alanine in Figure 4a are only 12 and 15 Hz, significantly narrower than previously reported under slightly different experimental conditions.[7] The C^{α} line widths are so narrow that a 1:2 doublet of approximately 20 Hz can be clearly observed. The doublet arises from the residual dipolar splitting (RDS) (^{14}N,^{13}C), also known as second-order quadrupole-dipole cross term.[14–18] This dipolar splitting is not completely eliminated by MAS because the large quadrupular interaction of the ^{14}N nucleus tilts the axis of quantization of the ^{14}N spin away from the direction of the static magnetic field, so that the angular dependence of the interaction cannot be averaged out by spinning at the magic angle.[14–16] In ^{15}N enriched L-alanine by contrast (Figure 4d), the dipolar ^{15}N-^{13}C coupling is averaged to zero, and the C^{α} line collapses to a singlet.

The C^{α} resonance of natural-abundance glycine in Figure 5a has a slightly asymmetric peak (full width at half-height 48.6 Hz) tailing towards high frequency which can be ascribed to an ill-resolved RDS(^{14}N,^{13}C). The C′ carbon shows a symmetric resonance with a line width of 30.5 Hz. Selective enrichment of the C^{α} atom (Figure 5b) results in a splitting of the C′ resonance into a doublet of 53 Hz arising from the scalar coupling $^{1}J(C',C^{\alpha})$. The ability to resolve this doublet strongly depends on the accurate setting of the magic angle, since $\Delta\theta \neq 0$ leads to a residual CSA interaction (it was determined by Ye et al. that $\delta_{CSA} = -71$ ppm[13]). The line widths of the C′ and C^{α} resonances in glycine are not significantly affected by ^{13}C enrichment of the C^{α} site.

The observed line widths $\Delta\nu = 1/(\pi T_2^*)$ can be compared with the limiting homogeneous line widths $\Delta\nu' = 1/(\pi T_2')$.[7,19,20] The limiting homogeneous line widths provide a good criterion to evaluate the performance of proton decoupling.[7] The T_2' values reported in Table 1 were determined from the mono-exponential decays of spin echoes. The defocusing and refocusing intervals τ were chosen to be multiples of the rotor period, while the longest delay was $2\tau = 150$ ms. For cholesteryl acetate, the T_2' time constants and corresponding line widths $\Delta\nu' = 1/(\pi T_2')$ are listed in Table 1. The longest $T_2' = 3.6$ s was found for the carbonyl carbon, corresponding to $\Delta\nu' = 0.09$ Hz. Such long T_2' time constants make it possible to design very complex pulse sequences, well beyond those currently used in liquid-state NMR spectroscopy. The width of 3 Hz observed under similar conditions for adamantane ($\nu_r = 10$ kHz) is believed to be mostly due to B_0 inhomogeneity. The experimental width for the carbonyl carbon signal being 3.9 Hz, the difference of 3.9–0.09–3 = 0.81 Hz must therefore be due to magnetic susceptibility effects, to a chemical shift dispersion arising from structural disorder, and to temperature gradients.[19] For the protonated carbon atoms in cholesteryl acetate, T_2' is much shorter, which indicates that the observed width is due in part to incomplete proton decoupling. In $^{13}C^{\alpha}$-enriched glycine, $T_2'(C^{\alpha}) = 52$ ms, while in natural abundance L-alanine $T_2' = 403$ for C′, 73 for C^{α}, and 69 ms for C^{β}. To evaluate the performance of decoupling with a slight miss-set of the angle, spin-echo decay curves were recorded for $\Delta\theta = 0.134°$. The resulting T_2' values in L-alanine were found to be 466 for C′, 75 for C^{α}, and 69 ms for C^{β}, that is, there is a slight increase for the C′ resonance, and little effect for C^{α} and C^{β}.

Friction results in sample heating and temperature gradients across the sample, which leads to line broadening.[21–24] Nuclei such as ^{207}Pb have isotropic shifts with a pronounced temperature dependence.[24] The ^{207}Pb MAS spectra of $Pb(NO_3)_2$ (Figure 6) show that different spinning

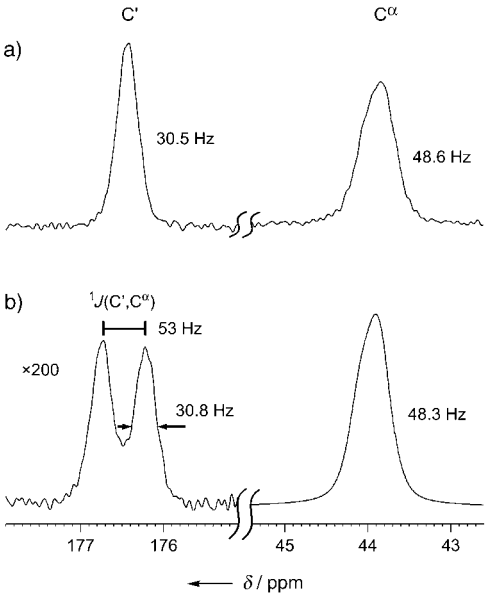

Figure 5. ^{13}C CPMAS spectra of a) natural-abundance glycine and b) glycine enriched in ^{13}C at the C^{α} position, recorded at the magic angle. The $^{1}J(C',C^{\alpha})$ coupling in (b) can be read directly from the spectrum. The spectra result from averaging 640 transients with a recycle interval of 2 s and a CP contact time of 1 ms. The acquisition time was 0.136 s. No line broadening was applied.

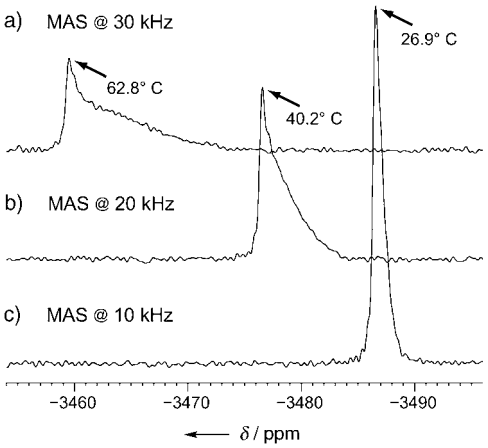

Figure 6. ^{207}Pb MAS spectra of $Pb(NO_3)_2$ recorded at different spinning rates, which lead to different temperature gradients across the sample. The spectra result from averaging of 320 transients with a recycle interval of 5 s.

Table 2: Differences ($\Delta\delta_{iso}$) between isotropic chemical shifts of ^{13}C of cholesteryl acetate observed at temperatures of 39 °C ($\delta_{iso}(39°)$) and 17 °C ($\delta_{iso}(17°)$).[a]

δ_{iso} [ppm][b]	170.90	141.47	121.78	73.49	52.71	41.57	37.48	34.27	32.84	28.48	21.32	13.89
$\Delta\delta_{iso}$ [Hz][c]	22	31	21	34	29	33	28	19	31	27	28	30
$\Delta\delta_{iso}/\Delta T$ [ppm/°][d]	0.010	0.014	0.010	0.015	0.013	0.015	0.013	0.009	0.014	0.012	0.013	0.014

[a] Nominal temperatures measured by a thermocouple in the air flow near the rotor. [b] The isotropic shifts in the first row correspond to 39 °C. [c] $\Delta\delta_{iso} = \delta_{iso}(39°) - \delta_{iso}(17°)$. [d] Derivatives $\Delta\delta_{iso}/\Delta T$ in ppm/degree. Partial assignments can be found in the work of De Paëpe et al.[12]

rates affect the temperature gradients. The absolute temperature is calibrated from the chemical shift difference of the two proton resonances in liquid methanol, which can be spun up to 30 kHz.[25] The temperature distribution across the sample is asymmetric, and its range increases from about 3° at 10 kHz to as much as 17° at 30 kHz. To estimate contributions from temperature gradients to line widths of ^{13}C spectra obtained under similar conditions, we recorded spectra of cholesteryl acetate and L-alanine in natural abundance at two different nominal temperatures. All line widths remain the same, but various downfield shifts are observed. The differences in chemical shifts $\Delta\delta = \delta_{iso}[39°] - \delta_{iso}[17°]$ for cholesteryl acetate are given in Table 2. For L-alanine $\Delta\delta = 20$ for C′, 30 for C^{α}, and 25 Hz for C^{β}. Clearly, the temperature gradient in the samples of cholesteryl acetate and L-alanine are much smaller than in $Pb(NO_3)_2$, otherwise the lines would be much broader and feature an asymmetry like that in Figure 6. Materials have different densities, heat capacities, electric and thermal conductivities, and dielectric properties, all of which can affect heating under sample rotation and RF irradiation. It is conceivable that the combined effects of mechanical friction and RF heating lead to a (fortuitous) reduction of the temperature gradients across our samples. The deliberate reduction of temperature gradients should lead to further improvements in resolution.

To summarize, we have shown that the accuracy of the adjustment of the magic angle is critical to obtain high-resolution MAS NMR spectra. The main contribution to the line widths in ^{13}C spectra recorded with a slight miss-set of the angle is due to residual CSA interactions. For quaternary carbon atoms, the increase in line widths is therefore greater than for proton-carrying carbon atoms. For the latter, the performance of heteronuclear proton decoupling is more critical, although it is not significantly affected by a small miss-set of the angle. Nevertheless, the observed line widths are still larger than the homogeneous limit given by the time-constant T_2' of spin echo decays. The remaining line widths are believed to be mainly due to magnetic susceptibility effects and to chemical shift dispersion arising from structural disorder or temperature gradients. Such effects can limit resolution at higher fields.

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