J|A|C|S COMMUNICATIONS

Published on Web 03/31/2010

Breaking the T_1 Constraint for Quantitative Measurement in Magic Angle Spinning Solid-State NMR Spectroscopy

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One of the outstanding advantages of NMR spectroscopy is its capacity of providing quantitative measurement for systems from molecular to macroscopic scales, compared to the other spectroscopic methods.[1] This rather unique property of NMR is important for measuring the populations of different sites, relative concentrations of interesting components, multiscale structures and dynamics, etc. The quantitative NMR experiments can be performed by the single pulse (SP) scheme, but generally it is time-consuming because the recycle delay which is typically 5 times the longest T_1 of the observed nuclear spins can be rather long,[2] unrealistic especially for the nuclear spins with a relatively long T_1 such as in the case of ^{13}C, ^{29}Si, and even ^{31}P.

This barrier of T_1 constraint still cannot be surmounted in solid-state NMR (ssNMR) by conventional methods. Recently, several improved cross-polarization (CP)[3] pulse schemes have been proposed for quantitative measurement in magic-angle-spinning (MAS)[4−6] ssNMR where the experimental time is saved significantly because it is not determined by the spin−lattice relaxation time of the observed spin, but rather by that of the abundant nucleus (i.e., ^{1}H, ^{19}F).[7−10] Therefore, the recycle delay (rd) has only to satisfy $\tau_{rd} > 5T_1(^1\text{H})$. However, the optimization of the recycle delay ($>5T_1$) as well as CP matching conditions is still required before starting the quantitative NMR experiment.

Here, we present quantitative ssNMR experimental techniques that can break the conventional T_1 constraint, as shown in Figure 1. A mixing period with broad-band homonuclear recoupling is introduced in CP/MAS and SP/MAS experiments for achieving quantitative CP and SP NMR spectroscopy, respectively. In the present work, a DARR recoupling technique[11] is performed during the mixing time, where nonuniform spin magnetization is redistributed under the reintroduced hetero- and homonuclear dipolar couplings that eventually drive the low-γ spin system to a quasi-equilibrium state so that spin magnetizations truthful to the populations of the respective spin sites are achieved. With these new schemes, quantitative NMR spectra can be acquired with a recycle delay significantly shorter than that mandated by T_1 of the low-γ or high-γ nuclei, in labeled or natural abundance samples.

Figure 2 shows ^{13}C MAS spectra of ^{13}C isotope-enriched L-tyrosine spun at 14 kHz acquired by a single 90° pulse (a), conventional CP (b, c), and QUCP (d, e), respectively. Compared with the ^{13}C SP/MAS spectrum with an optimized recycle delay (Figure 2a), the enhancement factors of different ^{13}C sites from CP and QUCP spectra can be calculated for each individual peak, which are plotted in Figure 2f and 2g, respectively. It can be seen

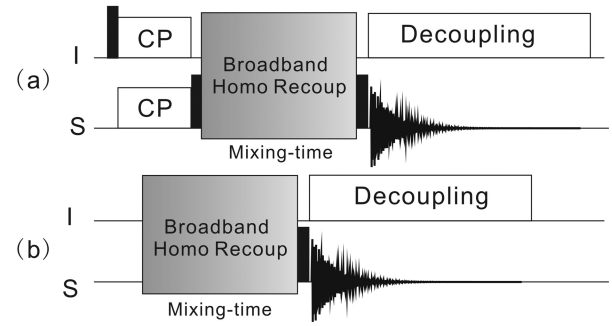

Figure 1. Pulse sequences for QUCP (a) and QUSP (b) experiments. Solid rectangles denote $\pi/2$ pulses.

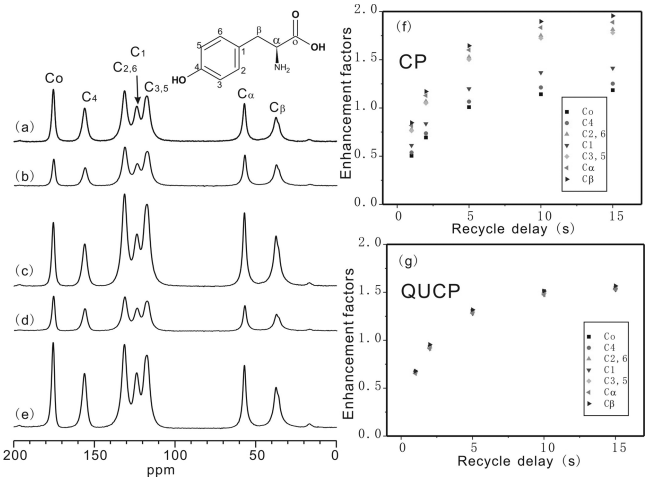

Figure 2. ^{13}C MAS spectra of ^{13}C, ^{15}N-labeled L-tyrosine recorded with a single pulse (a), CP (b, c), and QUCP (d, e). The spectra are plotted on the same amplitude scale, and 16 scans were accumulated for each spectrum. The recycle delay was 500 s for (a), 1 s for (b, d), and 15 s for (c, e). For QUCP experiments, the mixing time with DARR irradiation was 1 s, and the irradiation intensity was 14 kHz. The other experimental parameters were the same as the corresponding CP experiments. The enhancement factors for CP and QUCP experiments are plotted in (f) and (g), respectively.

that uniform enhancement cannot be obtained by the conventional CP technique, and the ratio between C_β and C_o reaches 1.7, even with a long recycle delay. However, for QUCP experiments, no matter what the recycle delay is, the uniform enhancement with a deviation of ±0.02 is nevertheless obtained. It can be seen that the T_1 constrained recycle delay is not necessary any more in the quantitative NMR experiments by the QUCP scheme and the uniform enhancement can always be achieved, so long as the mixing time is sufficiently long. For a quantitative NMR spectrum with

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10.1021/ja909550f

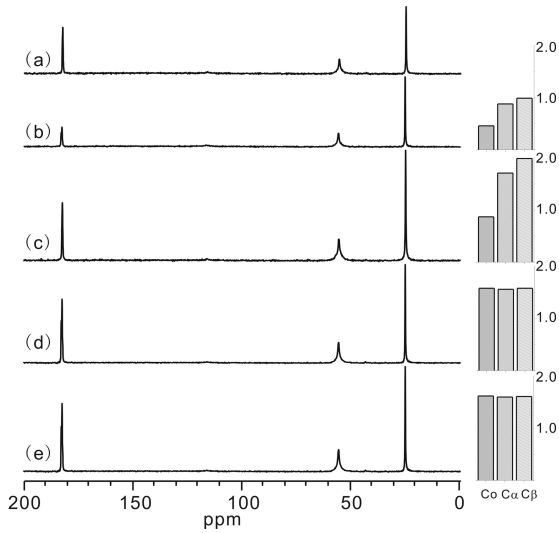

Figure 3. ^{13}C SP (a, b) and QUSP (c, d, e) NMR spectra of natural abundance DL-alanine spun at 14 kHz. The spectra are plotted on the same amplitude scale, and 200 scans were accumulated for each spectrum. The recycle delays were 200, 15, 5, 5, and 10 s for (a)−(e), respectively. For QUSP experiments, mixing time with DARR irradiation of 10, 20, and 20 s were used for (c), (d), and (e), respectively. The RF field intensity was set equal to MAS frequency, 14 kHz. The calculated recovery factors are plotted in the right bar charts.

truthful intensities, the efficiency gain can be defined as the ratio of the time required by the conventional SP experiment versus that by the QUCP scheme. It can be seen that a remarkable efficiency gain of 167.5 is obtained by the QUCP scheme with a recycle delay of 1 s. It demonstrates that not only are all spin magnetizations enhanced uniformly but the experimental time also is reduced significantly. Notably, the present scheme can break the T_1 constraints in quantitative NMR measurements. The detailed experimental and theoretical results (see Table S1 and Figure S1) demonstrate that higher efficiency gain can be obtained by the QUCP scheme with a relatively short recycle delay.

As reported previously,[10] the QUCP scheme is also suitable for rare nuclear systems in natural abundance. Quantitative measurement is demonstrated in detail for ^{13}C QUCP experiments with different recycle delays on natural abundance DL-alanine (see Figure S2 and Table S2). Because the needed RF field strength of DARR irradiation is usually less than 1/5 of the maximum, a long DARR mixing time (up to tens of seconds) is safe for NMR hardware in QUCP. In addition, corroborative results have been obtained from ^{31}P QUCP experiments on rehydrated calcined $AlPO_4$-41 (see Figure S3 and Table S3).

Furthermore, the quantitative measurements by QUSP scheme breaking T_1 constraints have also been accomplished. ^{13}C QUSP experimental results of uniformly labeled L-tyrosine show that the uniform recovery factors with a deviation of $\pm$0.003 for nonequivalent ^{13}C sites in QUSP spectra can readily be achieved no matter what the recycle delay is and an efficiency gain of $\sim$5 can be achieved with relatively short recycle delays. A higher efficiency gain (>5) can be obtained by combining with the small flip-angle experiment (see Figures S4−5). Figure 3 shows ^{13}C MAS spectra of natural abundance DL-alanine spun at 14 kHz acquired by single 90° pulse (a, b) and QUSP (c−e) schemes. An optimized recycle delay of 200 s was used to record a quantitative MAS spectrum, as shown in Figure 3a. From the calculated recovery factors as indicated in right bar charts in Figure 3, it can be seen that the methyl carbon magnetization can recover completely within a period of 15 s due to its fast spin−lattice relaxation rate, but carbonyl and methenyl carbons cannot. When DARR irradiation is applied, the recovery factors of methenyl and methyl carbons are larger than 1.0 due to NOE enhancement. Meanwhile, an increase in the recovery factor of carbonyl carbon can be obtained which is attributed to polarization transfer from two other carbons. When a longer mixing time (20 s) with DARR irradiation was used, polarization transfer among the three carbon sites would result in a quasi-equilibrium state with uniform recovery factors, as shown in Figure 3d, in the range of 1.57 ± 0.01. Similarly, a quantitative ^{13}C MAS spectrum with a uniform recovery factor in the range of 1.61 ± 0.01 can be obtained for a QUSP experiment with a recycle delay of 10 s. Taking a τ_{DARR} of 20 s into account, the efficiency gain of the QUSP experiment can be calculated, which is 12.5 and 10.7 for the experiment with a recycle delay of 5 and 10 s, respectively. Although a longer mixing time is required due to NOE, it is able to produce a sufficient efficiency gain (>5.0). It should be noted that the small flip-angle would result in a lower efficiency gain when NOE enhancement is present (see Table S4).

In summary, we proposed that the T_1 constraint on the recycle delay could be broken in quantitative MAS ssNMR. During the mixing time, the polarization among nonuniformly enhanced or recovered spin magnetizations is driven by the reintroduced dipolar interactions, toward a quasi-equilibrium state so that the nuclear magnetizations correspond to the spin site populations. Specifically, the experimental schemes have been demonstrated to be applicable for obtaining quantitative CP/MAS and SP/MAS spectra no matter what the recycle delay is. More noticeably, high efficiency gains can be obtained with relatively short recycle delays. These schemes are suitable for a labeled system as well as a rare nuclei system in natural abundance. Generally, the QUCP scheme is more suitable for the systems with abundant spins, and the QUSP scheme is applicable for the systems without abundant spins (^{1}H) or with invalid cross-polarization. Alternative homonuclear recoupling techniques can be performed during the mixing time (see Figures S6−8). In addition, the present approach is expected to have great potential in multidimensional correlation spectroscopy. These possibilities are under exploration.

Supporting Information Available: Experimental details; More QUCP and QUSP NMR results; analysis of the efficiency gain by QUCP and QUSP; comparison and combination of QUCP/QUSP schemes with the small flip-angle technique. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (20933009, 20921004, and 20773159), the National Basic Research Program of China (2009CB918600), and National Science Council of the Republic of China (S.D., Contract Nos. NSC-96-2752-M-110-001-PAE and NSC-97-2752-M-110-001-PAE).

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JA909550F