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Spin Counting with Fast MAS

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A novel magic angle spinning (MAS) multiple-quantum spin counting experiment based on the C7 recoupling sequence of Lee *et al.* is described. In contrast to previous approaches the new experiment is applicable at fast MAS rates and can be used to follow the multiple-quantum excitation dynamics with fine time resolution. The new method is illustrated by experiments on adamantane at spinning speeds comparable to the nonspinning dipolar linewidth. The sizes of the clusters of dipolar-coupled spins measured using the new experiment are compared to those obtained using the original nonspinning spin counting technique of Baum *et al.* © 1999 Academic Press

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Multiple-quantum transitions in restricted scalar-coupled systems of spin-$\frac{1}{2}$ nuclei are often excited during the course of solution-state NMR experiments in order to simplify complicated spectra and to determine coupling topologies. In solid-state NMR the straightforward combination of these experiments and magic angle spinning (MAS) has proved useful for structural studies of zeolites based on double-quantum spectroscopy of dilute pairs of scalar-coupled silicon-29 nuclei (*1*). Until recently, applications to dipolar systems were essentially confined to the wideline "spin counting" experiments of Baum *et al.,* which follow the multiple-quantum excitation dynamics in an attempt to extract information about the size of localized clusters of coupled spins (*2*). Spin counting has been used to study the structure of amorphous silicon and carbon, as well as the distribution of adsorbate molecules in zeolites and of additives in polymer films (*3*). The incorporation of MAS is not so straightforward in the dipolar case, due to the reversal of the multiple-quantum excitation dynamics during the second half of the rotor period (*4*). An early solution due to Meier and Earl involved synchronizing phase-shifted variants of the excitation sequences used by Baum *et al.* with the rotor in order to compensate for the effects of spinning (*5*). The past few years have seen the advent of "recoupling" sequences designed to reintroduce MAS-averaged dipolar couplings between spin-$\frac{1}{2}$ nuclei (*6*). This advance has prompted the development of solid-state MAS-based counterparts to the solution-state multiple-quantum applications described above. These include double-quantum filtration to remove background signals from the carbon-13 spectra of labeled biomolecules (*7*) and double-quantum spectroscopy (*8*) to study the structures of phosphate materials (*9*) and the dynamics of polymer melts (*10*).

However, with one exception (see below) high-order coherences have not been observed in straightforward MAS multiple-quantum experiments to date. Problems arise because many recoupling sequences suffer from low multiple-quantum excitation efficiencies in powder samples, since the strength of the recoupled dipolar interaction varies significantly with molecular orientation. In addition, some sequences operate correctly only in the limit of very short radio frequency pulse lengths relative to the rotor period, a condition which becomes difficult to satisfy as the MAS rate is increased. Finally, the presence of resonance offset and chemical shift anisotropy interferes with recoupling and further reduces excitation efficiency. A degree of compensation for these effects can be obtained in a straightforward fashion by concatenation of phase-shifted variants of the basic sequences, but this leads to unacceptably long cycle times. These restrictions pose a considerable limitation on the potential utility of recoupling sequences in spin counting applications.

Recently, 10-quantum coherence has been excited in proton MAS experiments on adamantane by Ba and Veeman (*11*), using the method of Meier and Earl. However, for the reasons described above, those experiments sampled the excitation dynamics with extremely coarse time resolution at MAS rates significantly smaller than the nonspinning linewidth. Tomaselli *et al.* (*12*) have detected 8-quantum coherence in proton MAS experiments on calcium formate at 16 kHz, a rate comparable to the nonspinning linewidth. However, in that work the multiple-quantum coherences were generated during carbon-13 detected coherence echo experiments. In this paper we present proton multiple-quantum spin counting experiments in which high-order coherences are excited in a straightforward fashion. We demonstrate that high-order coherences can be excited at MAS rates comparable to the nonspinning linewidth and that the growth of the multiple-quantum intensities can be measured with very fine time resolution. Finally, we show that the

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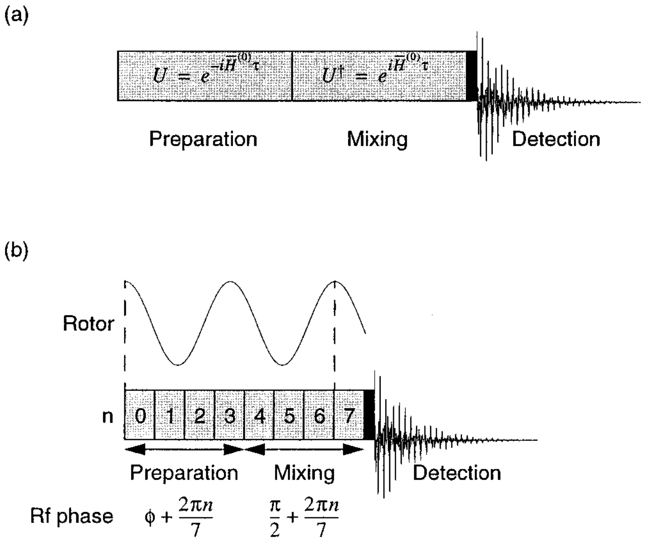

FIG. 1. Pulse sequence used to follow multiple-quantum excitation dynamics. (a) shows the overall phase-incremented experimental scheme, divided into preparation, mixing, and detection periods. During the preparation and mixing periods (shaded boxes), which are of equal duration τ, a suitable recoupling sequence operates. The propagators U and $U^{\dagger}$ acting during the two periods and the apparent time reversal in the mixing period are indicated. The reconversion sequence is followed by a $\pi/2$ pulse (filled box) to excite observable transverse magnetization. (b) shows details of the C7-based experiments used in this paper. The shaded boxes represent the cyclic C segments used for excitation and reconversion, timed so that seven occupy two rotor periods, with successive phase shifts of $2\pi/7$. Note that the index n which determines the phase of a given segment according to the requirements of the C7 sequence runs continuously through both preparation and mixing periods. Segments in the preparation period have an additional phase shift ϕ which is incremented in small steps $\Delta\phi$ in successive experiments and acts as the variable in the Fourier transformation used to generate the spectra. Segments in the mixing period have an additional constant phase shift of $\pi/2$.

excitation dynamics are essentially identical to that observed for nonspinning samples.

The experiments described here follow the phase-incremented scheme (Fig. 1a) of Shykind *et al.* (*13*), which is an efficient method for investigating multiple-quantum excitation dynamics. Multiple-quantum coherences are excited during a preparation period by application of a suitable recoupling sequence and are subsequently reconverted to longitudinal magnetization. In order to separate the multiple-quantum coherences of different order p, a series of experiments is performed for which the phase ϕ of the excitation sequence is incremented by an amount $\Delta\phi$. In this way, coherence of order p acquires an additional phase shift of $p\Delta\phi$ for each experiment. Hence, Fourier transformation of the signal $S(\phi, t = 0)$ with respect to ϕ results in a spectrum in which multiple-quantum intensities appear as delta-functions at artificial frequency offsets proportional to p. The phase increment $\Delta\phi$ must be set small enough to avoid aliasing of high-order coherences. In practice, several copies of the resulting data set are concatenated and the result is multiplied with a line broadening function prior to Fourier transformation in order to produce a more pleasing lineshape. Odd-order coherences can be removed if desired by coadding signals $S(\phi, t = 0)$ and $S(\phi + \pi, t = 0)$, but in the present case this procedure has not been followed, in order to emphasize the quality of the experimental results obtained.

Multiple-quantum excitation is achieved in this work by the C7 recoupling sequence (*14*) of Lee *et al.* This sequence comprises seven cyclic pulse segments (denoted C) timed to occupy two rotor periods in total, with neighboring C segments differing in phase by $2\pi/7$ (Fig. 1b). One simple C segment is a $2\pi_0 2\pi_\pi$ cycle where the subscripts represent the relative phases of the two pulses within the segment. This choice implies a relationship between the rotor frequency and the field strength $\omega_1 = 7\omega_R$. To zeroth order the effective C7 Hamiltonian for a dipolar-coupled pair of spin-$\frac{1}{2}$ nuclei has been shown (*14*) to be

$$\bar{H}^{(0)} = \omega_{C7} T_{2+2} + \omega_{C7}^{*} T_{2-2}$$

$$\omega_{C7} = \frac{-343(i + e^{i\pi/14})}{520\pi\sqrt{2}}\,\chi \sin 2\beta \exp(-i\gamma),$$

where $T_{2\pm 2}$ is an irreducible spherical tensor operator, β and γ are two of the Euler angles which relate the molecular frame to the rotor frame, and χ is the dipolar coupling constant which depends on internuclear distance r

$$\chi = -\left(\frac{\mu_0}{4\pi}\right)\frac{\gamma^2\hbar}{r^3}.$$

Apart from a phase shift, the spin part of the C7 effective Hamiltonian is identical to the double-quantum dipolar Hamiltonian used for nonspinning multiple-quantum excitation by Baum *et al.* (*2*). The sign of the effective Hamiltonian must be changed in order to obtain the apparent time reversal necessary for reconversion without phase distortion. This is simply achieved by application of an extra $\pi/2$ phase shift to all the C7 pulses in the mixing period. In the limit of fast MAS where the spinning rate and the radio frequency field strength are much greater than the nonspinning proton linewidth, the effective double-quantum dipolar Hamiltonian and its time-reversed counterpart are valid for the extended systems probed by spin counting experiments.

It should be noted that in common with all recoupling sequences, C7 reintroduces a scaled orientation-dependent dipolar coupling. A convenient measure of the scaling is obtained by consideration of the orientationally averaged norm of the coupling $\|\omega_{C7}\|$ which is given by

$$\|\omega_{C7}\|^2 = \frac{1}{4\pi}\int_0^{2\pi} d\gamma \int_0^{\pi} \sin\beta d\beta\, \omega_{C7}\omega_{C7}^{*}.$$

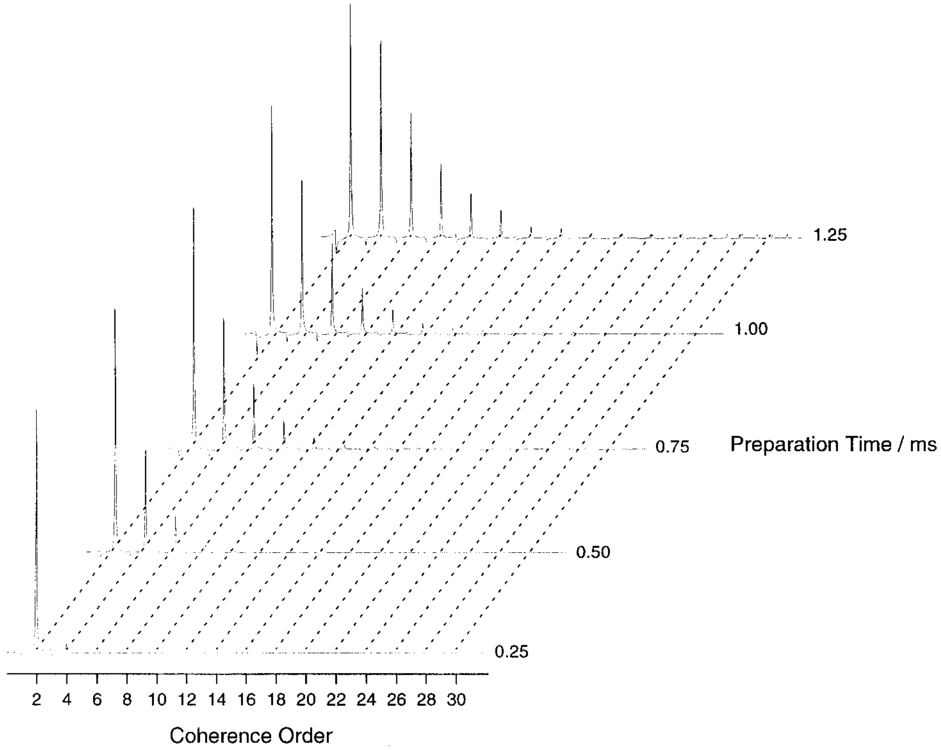

FIG. 2. Proton phase-incremented multiple-quantum experiments for adamantane. The proton resonance frequency was 300.07 MHz, and the MAS rate was 8 kHz. Complete cycles of the C7 recoupling sequence were used for excitation and reconversion, with preparation times ranging from 0.25 to 1.25 ms. There were 64 values of the phase ϕ with a phase increment of 5.625°. Note the excitation of 16-quantum coherence at the longest preparation time.

The norm of the coupling for the C7 sequence operating on an isolated pair of spin-$\frac{1}{2}$ nuclei is approximately 0.169 χ (*14*). In contrast to other sequences for C7 the scaling depends only on β, so that multiple-quantum coherences can potentially be created with high efficiency in a powder. In addition, deleterious resonance offset and radio frequency field inhomogeneity effects are removed to zeroth order over the individual C segments (*15*), while shift anisotropy terms are likewise canceled by the symmetry properties of the sequence.

A particular advantage of the approach described here is that the excitation dynamics can be studied with fine time resolution, regardless of constraints imposed by the pulse sequence cycle time and the rotor period. This is achieved by sampling the excitation dynamics with the preparation period set to nonintegral multiples of the overall cycle time. It has been demonstrated (*14*) that the evolution under the C7 effective Hamiltonian, which is theoretically valid only after whole cycles of the sequence, is a good approximation to the actual evolution for nonintegral numbers of C7 cycles, provided sampling is restricted to whole numbers of C segments. In order to take advantage of this property of C7 care must be taken with the relative phases of the C segments (*14*), as shown in Fig. 1b.

Figures 2 and 3 show results for the C7-based phase-incremented proton multiple-quantum experiment of Fig. 1 on adamantane at a Larmor frequency of 300.07 MHz at MAS rates of 8 and 16 kHz, respectively. The multiple-quantum excitation dynamics were sampled after whole numbers of C7 cycles, so that the preparation time ranged from 0.25 to 1.25 ms at 8 kHz and from 0.125 to 0.875 ms at 16 kHz. Double-quantum and a small amount of four-quantum coherence are excited even at the shortest preparation times, while higher orders are created as the number of cycles is increased. Up to 16-quantum coherence is clearly observed at longer preparation times and odd-order coherences which were not removed in the data processing are of negligible intensity. For comparable preparation times similar multiple-quantum intensities are obtained for the two MAS rates, although as expected efficiency is slightly improved at the faster MAS rate for which higher order error terms in the effective Hamiltonian are less significant. For adamantane, relaxation of the multiple-quantum coherences during the excitation and reconversion sequences was the main limitation on the maximum preparation time which could be used.

Figure 4 demonstrates the possibility of sampling with incomplete C7 cycles. The excitation dynamics are observed first after six C segments and subsequently after every second C segment with a MAS rate of 8 kHz and preparation times ranging from 0.214 to 1.071 ms. The multiple-quantum intensities in these experiments fall between those observed with complete cycles in Fig. 2, as expected. The observation of high-order coherences at longer preparation times and the

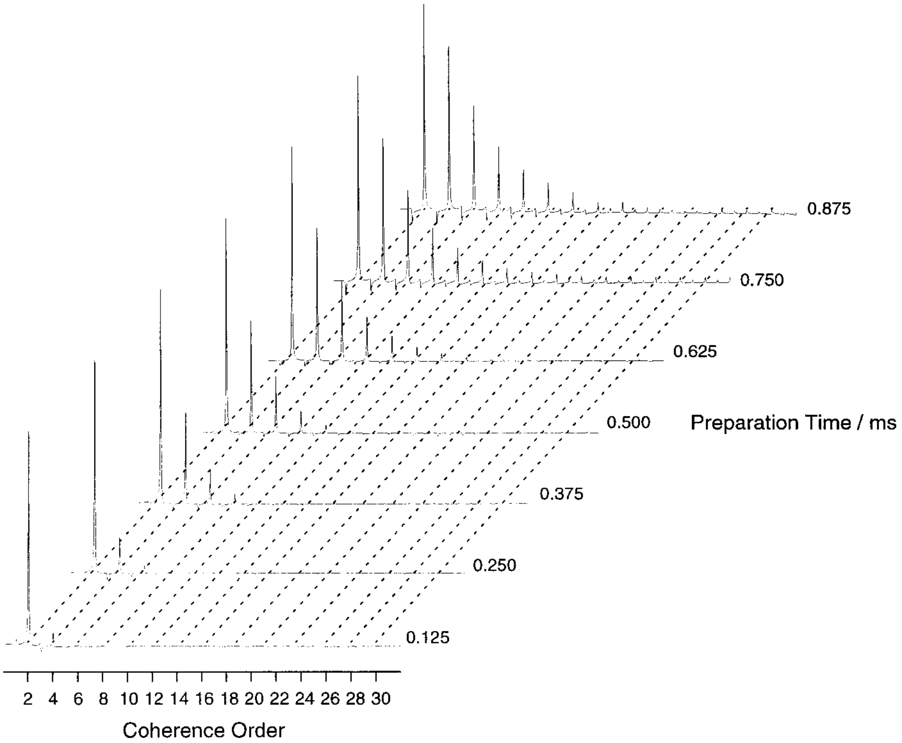

FIG. 3. Same as Fig. 2 but with a MAS rate of 16 kHz and preparation times ranging from 0.125 to 0.875 ms. The multiple-quantum intensities are comparable to those of Fig. 2 at corresponding preparation times.

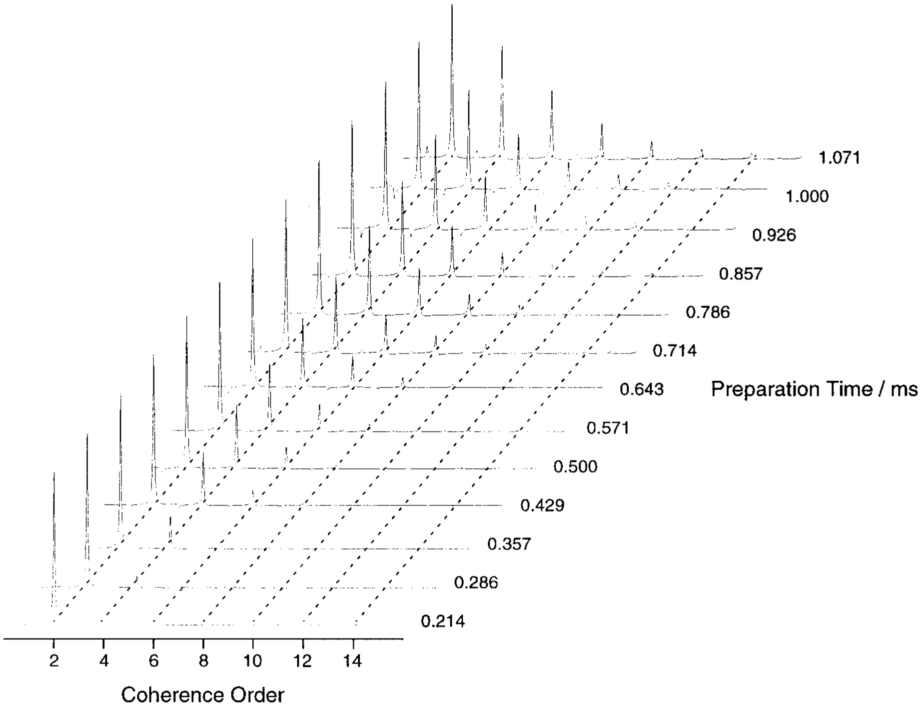

FIG. 4. Same as Fig. 2 but using incomplete cycles of the C7 sequence for excitation and reconversion with preparation times ranging from 0.214 to 1.071 ms. The multiple-quantum intensities are comparable to those of Fig. 2 at similar preparation times.

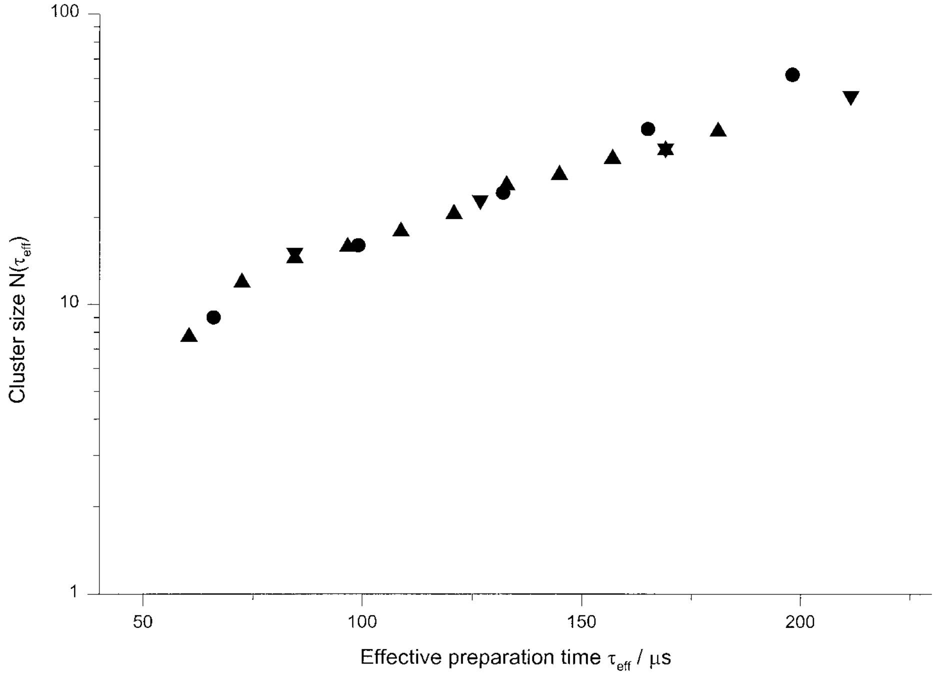

FIG. 5. Cluster size *N* measured as a function of effective preparation time (see text) for the data of Figs. 2 and 4, as well as a comparison with measurements made using the original nonspinning experiment. The coincidence of the points sampled after integral numbers of C7 cycles (▼) and those sampled after individual C segments (▲) should be noted. This gives an indication of the validity of the C7 effective Hamiltonian even after incomplete cycles. The good agreement between cluster sizes measured using the nonspinning and the MAS experiments is gratifying. For the nonspinning experiments excitation and reconversion were achieved with the pulse sequence described by Baum *et al.* (*2*) for preparation times ranging from 132 to 396 μs. As in the MAS experiments there were 64 values of the phase ϕ with a phase increment of 5.625°.

negligible odd-order intensities provide an experimental demonstration of the unexpected validity of the C7 effective Hamiltonian for incomplete cycles (*14*).

In the experiments described here the best multiple-quantum excitation efficiencies were observed with radio frequency field strengths of up to 25% less than $7\omega_R$, such that the C cycles contained pulses with flip angles of less than 2π. The origin of this improvement is under investigation, but it should be noted that high orders were still observed with the field strength set to the correct value. There was no improvement when C7 was replaced by a modified sequence (*15*) which removes error terms in the effective Hamiltonian arising from the combined effects of offset and inhomogeneity. This is expected for the proton experiments described here, in which resonance offsets are negligible.

Estimates of the effective size *N* of the dipolar-coupled spin cluster for a given preparation time can be extracted from the data by assuming that the intensity of p-quantum coherence is related to the number of different transitions of order p in a system of N spins. Simple combinatorial arguments (*2*) then suggest that for large clusters the multiple-quantum intensity distribution is approximated by a Gaussian of variance $N/2$. Hence, the experimental multiple-quantum intensities were fitted to a Gaussian intensity distribution of the form $A \exp((-p^2)/N)$, where A and N are adjustable parameters. The small spurious peaks observed at very high coherence orders were used to define the baseline for the measured intensities. Figure 5 shows the growth of the cluster size measured as a function of the preparation time for the 8-kHz MAS experiments of Fig. 2 (▼) and Fig. 4 (▲). To aid comparison with nonspinning measurements (see below) the cluster sizes are plotted against an effective preparation time $\|\omega_{C7}\|\tau/\chi$, which takes the scaling of the dipolar coupling into account. As expected the cluster size grows monotonically with increasing preparation time. The observed multiple-quantum excitation dynamics are similar to that observed by Baum *et al.* in nonspinning experiments on adamantane. The results of similar fits to data from nonspinning experiments identical to those described in Ref. 2 are also plotted in Fig. 5 (circles). The moderate scaling of the dipolar coupling imposed by the pulse sequence of Baum *et al.* was taken into account. The match between cluster sizes measured by MAS and nonspinning experiments as a function of effective preparation time is gratifying.

The experimental results presented in this paper demonstrate that high-order multiple-quantum coherences can be excited in solids spinning at MAS rates greater than the nonspinning linewidth using suitable recoupling sequences. This allows measurements of cluster sizes to be made with the high spectral resolution afforded by MAS. Spin counting experiments based on the C7 recoupling sequence have been shown to be particularly effective due to the favorable orientation dependence of the recoupled dipolar interaction, as well as good compensation for error terms. At long preparation times the effective size of the spin cluster is large, indicating that the C7 sequence effectively recouples the dipolar interaction. At these long times and with effective recoupling, the full multibody behavior characteristic of a nonspinning dipolar solid is evidenced by our results. A full account of the contrasting behavior of spin systems of arbitrary topology under fast MAS in the absence of a recoupling sequence is given elsewhere (*16*). A noteworthy feature of the experiments described here is the ability to sample the excitation dynamics with fine time resolution. This represents an advance over previous MAS spin counting experiments and is achieved by a combination of fast MAS and sampling after incomplete cycles of the C7 sequence. Further improvements in resolution can be achieved by combining these MAS experiments with cross polarization to a dilute heteronucleus such as carbon-13 (*11, 12*). Future applications of the experiments described here to high-order carbon-13 multiple-quantum filtration experiments in globally labeled biomolecules can also be envisaged.

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