

Elucidation of Dipolar Coupling Networks under Magic-Angle Spinning

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A variety of novel two-dimensional shift correlation experiments have been proposed recently (1) which allow connectivities between dipolar-coupled spins in solids to be elucidated with the high-resolution characteristic of magic-angle-spinning spectra (MAS). In order to reconcile the conflicting requirements of MAS and magnetization transfer through dipole–dipole couplings, these experiments incorporate recoupling sequences during a mixing period inserted between the evolution and detection times. The recoupling sequences reintroduce either a zero- or a double-quantum variant of the dipolar Hamiltonian in order to promote magnetization transfer.

Complementary information about dipolar connectivities is also available from a MAS double-quantum spectroscopy experiment of the type demonstrated by Geen *et al.* (2). This monitors the evolution of the double-quantum coherence generated by the recoupled dipolar Hamiltonian, resulting in a two-dimensional spectrum which measures the sum of the chemical shifts of the dipolar-coupled spins in the first dimension. In this paper, a new experiment is described which results in spectra which retain the easily interpretable shift-correlation format, while incorporating some of the benefits of the double-quantum experiment. The new method uses the recoupled dipolar Hamiltonian as the basis of a double-quantum filter (3) which replaces the mixing period of the magnetization-transfer experiment.

The magnetization-transfer, double-quantum-filtered transfer, and double-quantum spectroscopy experiments have been compared during an investigation of the phosphorus dipolar coupling networks in magnesium pyrophosphate. Several crystalline and glassy phosphates have been characterized previously by both magnetization-transfer and double-quantum spectroscopy methods (4). It is shown here that, while the three spectra differ considerably in appearance, the information which they contain about dipolar connectivities is similar. In addition, the relative utility of the three experiments in studies of globally carbon-13-labeled biomolecules and of phosphate materials is evaluated.

As shown in Fig. 1, the three experiments follow the conventional scheme for two-dimensional NMR spectroscopy, and each can be separated into four time periods. The

initial “preparation” period involves the excitation of the coherence, which is sampled during the subsequent “evolution” period. In the “mixing” period, the coherences which evolve in the first dimension are converted into transverse magnetization for acquisition during the final “detection” period. During appropriate periods of each experiment, a recoupling sequence is applied which generates a double-quantum dipolar average Hamiltonian of the type

$$H^{(0)} = \frac{\Omega^{12}}{2} (I_1^+ I_2^+ + I_1^- I_2^-),$$

where Ω^{12} is a function of both the magnitude and the orientation dependence of the recoupled interaction.

Under this Hamiltonian, the sum polarization $I_{1z} + I_{2z}$ is transformed to double-quantum coherence according to

$$\begin{aligned} \frac{1}{2} (I_{1z} + I_{2z}) &\rightarrow \frac{1}{2} (I_{1z} + I_{2z}) \cos(\Omega^{12}\tau) \\ &+ \frac{i}{2} (I_1^+ I_2^+ - I_1^- I_2^-) \sin(\Omega^{12}\tau), \end{aligned}$$

while the difference polarization $I_{1z} - I_{2z}$ is invariant (5). For a two-spin system, the sum polarization oscillates, while in a system of many mutually coupled spins, the oscillations are damped and eventually decay to zero; in both cases, however, there is a net redistribution of polarization amongst the coupling partners. In the experiments described here, the C7 recoupling sequence, recently developed by Lee *et al.* (6), was used. The C7 average Hamiltonian for a pair of dipolar-coupled spins subject to chemical-shift anisotropy and resonance offset is a mixture of that given above and the orthogonal double-quantum operator $-(i/2)(I_1^+ I_2^+ - I_1^- I_2^-)$, but the effect of this modification is simply to shift the phase of the double-quantum coherence created.

Compensation for chemical-shift anisotropy, resonance offset, and radiofrequency-field inhomogeneity by the C7 sequence occurs on a rapid time scale which is a fraction of the overall cycle. This feature, coupled with a favorable orientation dependence of the recoupled dipolar interaction,

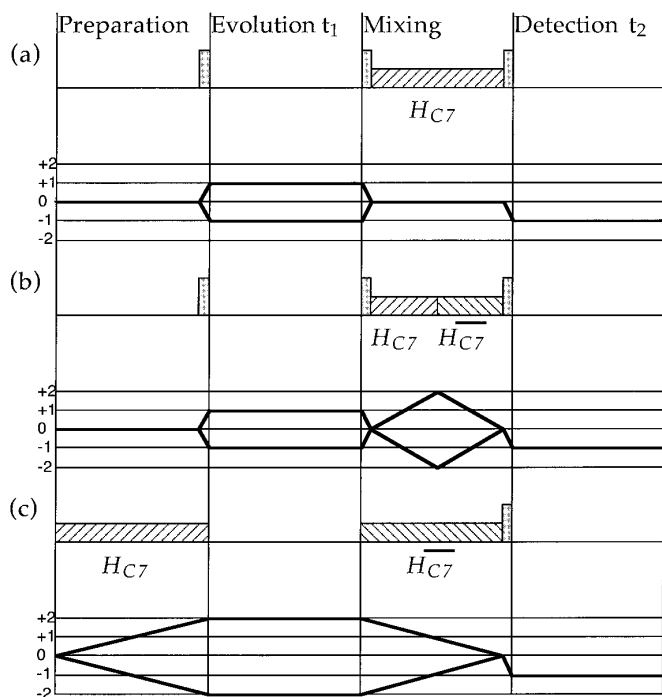


FIG. 1. Pulse sequences and coherence-transfer pathways associated with the three experiments described in the text: (a) magnetization-transfer experiment, (b) double-quantum-filtered magnetization-transfer experiment, (c) double-quantum spectroscopy experiment.

has been shown to maximize the experimental efficiency of multiple-quantum generation (6, 7). In order to eliminate higher-order contributions to the overall effective Hamiltonian, the measurements are performed with magic-angle spinning at a rate greatly exceeding the range of frequencies in the nonspinning spectrum. This precaution is an essential prerequisite for the correct operation of dipolar recoupling sequences in multispin systems (8) and is necessary here because the phosphorus spins constitute a mutually coupled network. It should be noted that this requirement may be necessary in similar experiments on biomolecules with a high degree of isotopic enrichment with, for example, carbon-13 or nitrogen-15. Only in systems of truly isolated dipolar interactions can this condition be relaxed; such an arrangement is found in materials which are doubly enriched with carbon-13 or another dilute isotope (9). Nevertheless, double-quantum spectra have been analyzed quantitatively using this assumption to yield proton-proton distances (10).

The experiment shown in Fig. 1a is a magnetization-transfer shift-correlation scheme which commences with the preparation and evolution of single-quantum coherence. The mixing period starts with a 90° pulse so that the density operator at the start of the $C7$ sequence consists of polarization on each spin labeled with the corresponding chemical-shift frequency during the evolution time. The recoupled double-quantum Hamiltonian excites even-order multiple-

quantum transitions (11) while simultaneously redistributing the residual polarization amongst the coupling partners. The selection of a zero-quantum coherence-transfer pathway as shown ensures that only this residual polarization is detected as single-quantum coherence after a final 90° pulse. However, contributions from uncoupled spins unaffected by the $C7$ sequence are also detected. This results in additional intensity along the spectral diagonal which obscures a part of the required information about the coupling network.

The second scheme (Fig. 1b) is a novel variant of the first, which begins similarly with the preparation and evolution of single-quantum coherence. Multiple-quantum coherence is excited by the $C7$ sequence, but in the new experiment the mixing period is extended by an equal number of $C7$ cycles shifted in overall phase by 90° . This procedure ensures that a double-quantum Hamiltonian of opposing sign operates for the second half, reconverting to polarization the multiple-quantum coherences generated in the first. In this case, coherence-transfer pathways are selected so that, rather than originating from residual polarization, the magnetization which is finally detected is filtered through double-quantum coherence during the mixing period. This magnetization is detected at the chemical-shift frequency of any one of the coupling partners involved in the multiple-quantum coherence. The filtration is achieved by phase cycling the $C7$ cycles of the first half of the mixing period to select a change in coherence order of ± 2 and has the beneficial effect of removing the uninformative diagonal intensity due to uncoupled spins observed in the first experiment. It should be noted that spectra filtered through higher orders of multiple-quantum coherence could also be recorded by a modified phase cycle.

The third scheme (Fig. 1c) is a double-quantum spectroscopy experiment. As in the second approach, the resulting spectrum records magnetization that is passed through double-quantum coherence and then reconverted, thus eliminating any signals from uncoupled spins. However, this experiment differs significantly from the previous two, since double-quantum coherences are excited during the preparation period and sampled in the evolution time before reconversion. Therefore, the resulting spectrum measures double-quantum frequencies in the first dimension.

Magnesium pyrophosphate $Mg_2P_2O_7$ shows temperature-dependent polymorphism (12), in common with a range of inorganic pyrocompounds. The high-temperature β phase has a thortveitite structure with the magnesium ions approximately octahedrally coordinated by the terminal oxygen atoms of the pyrophosphate anions. The bridging oxygen atoms lie between the essentially close-packed layers of terminal ones with a linear P-O-P bridging linkage. The low-temperature α phase is a less symmetrical polymorph in which two of the unit-cell dimensions are approximately doubled and the bridging P-O-P bond angle is decreased to 144° . As a consequence, there are two distinct phosphorus

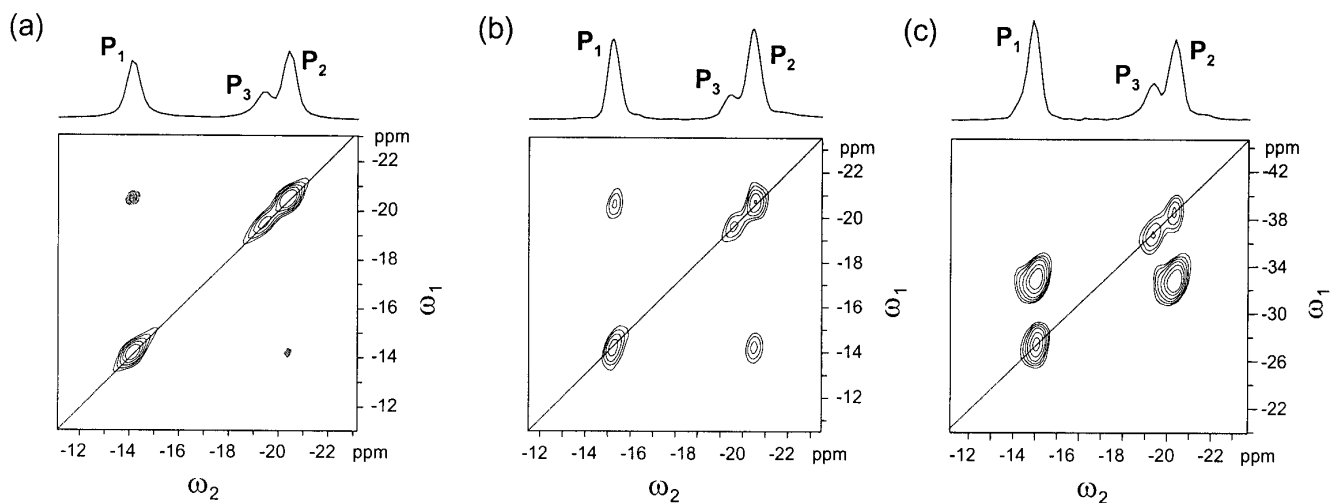


FIG. 2. Three two-dimensional phosphorus-31 spectra as described in the text: (a) magnetization-transfer spectrum, (b) double-quantum-filtered magnetization-transfer spectrum, (c) double-quantum spectrum. All experiments were recorded at a resonance frequency of 202.4 MHz with a MAS rate of 14 kHz. The coherence-transfer pathways shown were selected by suitable phase cycling, and the Marion–Wüthrich method for achieving two-dimensional pure-phase spectra was used in all three experiments. In (a) 8 cycles of the C7 sequence produced the recoupled double-quantum dipolar Hamiltonian H_{C7} , while in (b) and (c) 4 cycles of C7 each were used for the double-quantum excitation and reconversion processes with an overall phase shift of 90° during reconversion to produce the time-reversed Hamiltonian H_{C7} .

chemical shifts (labeled P_1 and P_2) in the α phase, while the β phase shows only a single resonance (P_3). The P_1 and P_2 phosphorus nuclei are located in different crystallites from the P_3 nuclei, so that a restricted set of the possible dipolar connectivities is expected.

The results shown in Fig. 2 clearly demonstrate that the three methods give similar information about the dipolar connectivities in $Mg_2P_2O_7$, although from spectra which, as expected, differ considerably in appearance. In the two magnetization-transfer experiments, (a) and (b), a dipole–dipole coupling between spins I and S is indicated by four peaks at offsets (Ω_I, Ω_I) , (Ω_I, Ω_S) , (Ω_S, Ω_I) , and (Ω_S, Ω_S) , whereas with double-quantum spectroscopy (c) there will be two peaks at $(\Omega_I + \Omega_S, \Omega_I)$, and $(\Omega_I + \Omega_S, \Omega_S)$. Superposition of such patterns from each of the four expected connectivities P_1 – P_1 , P_2 – P_2 , P_3 – P_3 , and P_1 – P_2 results in the observed spectrum. Because the double-quantum dipolar Hamiltonian perturbs only the initial sum-polarization state, the cross peaks in the original experiment which monitors the redistribution of this polarization are of opposite sign to the diagonal peaks, causing problems of mutual interference (Fig. 2a). In addition, this experiment results in uninformative intensity along the diagonal arising from uncoupled spins. Both of these deleterious effects are removed in the second and third variants of the experiment (Figs. 2b and 2c), in which the observed signal is filtered through double-quantum coherence.

The double-quantum spectrum (Fig. 2c) exhibits three peaks along the skew diagonal which have analogues in similar spectra recorded in isotropic phase for scalar-coupled spins only in cases of chemical (as opposed to magnetic)

equivalence. The preponderance of almost chemically equivalent mutually coupled spin pairs in phosphate materials indicates that double-quantum spectroscopy will be the method of choice for investigations of dipolar connectivities in similar inorganic compounds. This is because intensity at these frequency coordinates unambiguously verifies their existence in the double-quantum spectroscopy experiment (Fig. 2c), whereas diagonal peaks associated with chemically inequivalent coupled pairs will interfere in the double-quantum-filtered magnetization-transfer experiment (Fig. 2b). On the other hand, chemically equivalent coupling partners are rare in carbon-13-labeled biomolecules, even with 100% isotopic enrichment. In such a case, the double-quantum-filtered transfer experiment may be the method of choice because of its easily interpretable shift correlation format.

In principle, more detailed information about the dipolar-coupled network can be obtained from a quantitative analysis of the dynamics of the double-quantum excitation in these experiments. Simulations of the variation of cross-peak intensity as a function of mixing time for model spin systems with a range of coupling topologies are in progress and will be described in a subsequent publication. Computations of the dynamics of multiple-quantum excitation in nonspinning samples on systems of up to five spins have shown that, in certain favorable cases, the experiments can be used to derive information on the strength and relative orientations of the coupling tensors (13). It is anticipated that the same type of information can be obtained using the double-quantum experiments described here, but in a refined form because of the high spectral resolution due to the MAS and the addition of a second frequency dimension.

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