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Methylene-Only Subspectra in ^{13}C CPMAS Using a New Double Quantum Filtering Sequence

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Abstract

Methodology for the assignment of ^{13}C CPMAS spectra is still in its infancy. Previous methods of CPMAS spectral editing have utilized differences in the strength of the ^{13}C–^{1}H dipolar interaction or the rate and spin thermodynamics of crosspolarization from protons to carbon, to differentiate between quaternary, tertiary, and methylene carbons. We introduce a different approach, which is based on the fact that double-quantum coherence develops between the protons of a methylene group considerably faster than between most other proton spin pairs in an organic solid. We generate this coherence, filter it, convert it back to single quantum, and then crosspolarize selectively to carbon, followed by a short period of reversed crosspolarization to null out unwanted coherence generated from longer distance spin pairs. The sequence has been named DQCP. While the signal-to-noise of this method is poorer than ordinary CP, it is comparable to previous methods for generating methylene-only spectra, and the technique is straightforward and easy to implement.

Cross-polarization magic angle spinning (CPMAS) is the technique of choice to obtain narrow line carbon NMR spectra with high sensitivity in the solid state (*1–3*). Almost 20 years after the pioneering dipolar dephasing editing sequence by Opella and Frey (*4*), interpretation of ^{13}C CPMAS spectra in the solid state still remains a challenging nuisance, particularly when studying large systems and biologically relevant samples. In liquids simple scalar couplings are exploited in sequences such as DEPT, INEPT, and INADEQUATE (*5*), making the interpretation of carbon spectra of complex molecules routine. In contrast, with CPMAS in solids every effort is made to eliminate the major sources of line broadening—chemical shift anisotropy (CSA) and homo- and heteronuclear dipolar couplings—in order to obtain spectra with sharp lines. This dramatically reduces the amount of structural information contained in the CPMAS spectra.

Nevertheless, methods have been developed to overcome these limitations and to retrieve the lost information. For instance, the dipolar dephasing method cited earlier takes advantage of the difference in the rate of magnetization decay between carbons of different multiplicity. The carbons that relax fastest through dipole–dipole interactions, CH and CH_2, are virtually eliminated by introducing a delay period following the contact time. Another important characteristic of CH and CH_2 is the faster rate at which they crosspolarize, compared to quaternary or methyl carbons, and so using short contact times it is possible to enhance primary and secondary carbon signals (*6*). Other methods rely on the use selective polarization inversion schemes that successively add the desired signals and subtract the unwanted ones until a satisfactory spectrum is achieved. Many variations on the themes outlined above produced a number of useful spectral editing sequences (*7–12*). Another clever method called magic-angle spinning separated local field (MASSLF) allows assignment of the carbon moieties based upon their unique dipolar coupled patterns on a two-dimensional spectrum (*9*). Nor is the dipolar coupling the only variable at our disposal for spectral editing—CSA dephasing can be used to distinguish between nuclei based upon shift anisotropy rather than their multiplicity, as recently shown by Frydman and co-workers (*13*).

Our approach to spectral editing consists of the use of a new double-quantum (DQ) filtering pulse sequence to eliminate all but CH_2 resonances from the one-dimension ^{13}C CPMAS spectrum. The CH_2 selectivity derives from the fact that DQ coherence is generated faster on methylene carbons than on any other C–H spin combination. It was realized that, if the evolution and mixing times were chosen carefully, the magnetization could be transferred from the I spins to the directly bound S spin with minimal spin diffusion. Since methods were devised to detect higher orders of coherence (*14*) multiple quantum filters have been widely used to simplify both liquid and solid state NMR spectra (*15–20*). In earlier examples the CPMAS sequence was modified to include a filtering sequence in the S spin after mixing. On the contrary, in our sequence the filtering occurs at the beginning of the sequence, before the contact time. The result is a very simple sequence as compared to DQ-DRAWS, DRAMA, HORROR, and others, that performs comparatively well on a variety of organic samples.

The pulse sequences used in this work are shown in Figure 1. Both sequences employ the simplest possible three

pulse DQ filter, originally used in the double quantum COSY experiment (*15*). The first pulse generates single quantum transverse coherence, which then evolves into antiphase single quantum coherence during the period t_1. The second pulse then converts this antiphase coherence into double-quantum coherence (and to a lesser extent higher orders of coherence). The phase of the first pulse is cycled according to the relationship $\varphi = k\pi/2$ with $k = 0, 1, 2, 3$. In the second pulse a phase $\varphi + \psi$ is employed, and the ψ value is alternately 0 or π during an 8-step cycle so that at the end of the second pulse only pure double-quantum coherences remain. A third pulse immediately follows which converts the double-quantum terms in the density matrix back to antiphase single quantum.

In the first pulse sequences (Figure1a), this antiphase coherence evolves back into observable magnetization during t_2. The filtered magnetization is transferred to the rare spin during a short (and it is to be hoped, selective) period during which the Hartmann–Hahn condition is matched. Prior to mixing, the proton magnetization derives mostly from methylene protons plus some unwanted CH signal presumably due to a combination of spin diffusion from the CH_2 protons during t_2 and double quantum generation from nongeminal protons.

Initially we matched the t_1 and t_2 periods, reasoning that the optimal recovery of ordinary single-quantum coherence from antiphase coherence would be obtained with an evolution time which equaled the time allowed for evolution into that coherence. Serendipitously, however, we discovered that such matching was unnecessary, since such evolution persists during the Hartmann–Hahn condition, albeit at a scaled rate.

Because spin-diffusion and longer-range dipole interactions necessarily cause some signal to be produced for nonmethylene carbons, measures are necessary to remove these unwanted signals. A period of polarization inversion (PI) produces a spectrum that is virtually methylene-only. The t_1 and t_2 times, the CP and PI times, were optimized to obtain spectra of the best possible quality. First we determined the CP time by running a series of standard CPMAS experiments using increasingly shorter mixing times and observing the change in CH_2/CH intensity ratio for a tyrosine hydrochloride sample as shown in Figure 2. All ^{13}C spectra were recorded on a home-built spectrometer at 76.917 MHz and a proton 90° pulse of 4 μs. Chemical shifts are given in ppm from TMS. A 20 μs contact time, which is equivalent to the optimal mixing time used by Wu and Zilm (*10*), gave the highest CH_2/CH ratio, 1.3, and was used as an initial value for the optimization the DQCP sequence. Next we determined the t_1 and t_2 values for DQCP as shown in Figure3 for the same tyrosine hydrochloride sample. Equal values of 8 or 10 μs for both t_1 and t_2 give a slightly improved CH_2/CH ratio of 1.5 with respect to a normal CPMAS with short contact time (Figure 3a). The situation is improved greatly by reducing t_2 to 1 μs and holding t_1 to 8 μs; the CH_2/CH ratio is now between 2 and 2.5 (Figure 3b). Increasing the mixing time to 40 μs is detrimental for the CH_2/CH ratio that has a maximum value of only 1.5 at a 2 μs t_2 (Figure 3c). In the final optimization step the PI period was added; a 4 to 6 μs PI period removed the remaining CH peaks (Figure 3d).

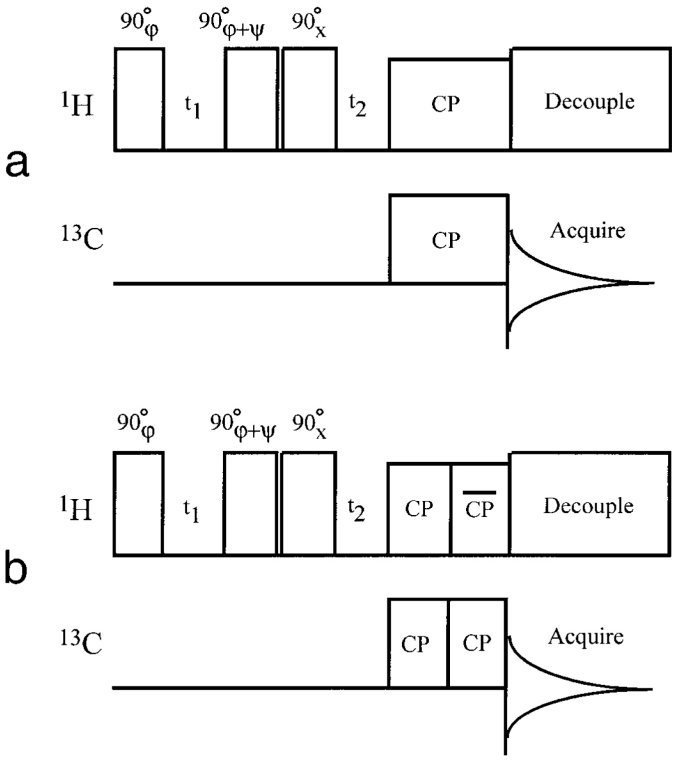

Figure 1. Pulse sequence diagrams. (a) The basic DQCP sequence. (b) The DQCPPI pulse sequence with added polarization inversion.

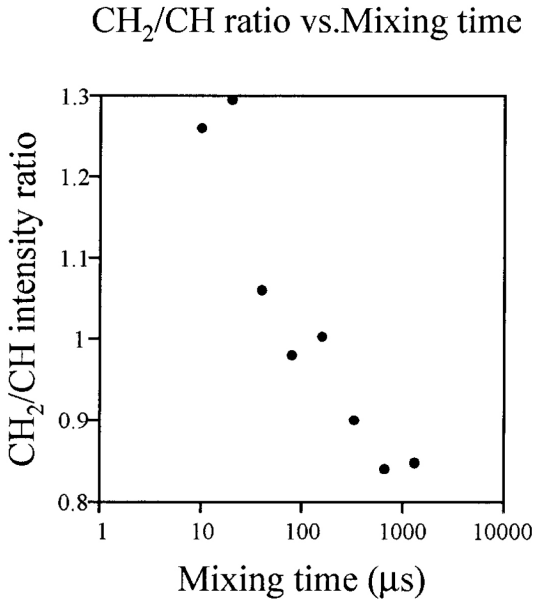

Figure 2. Normalized CH_2/CH intensity ratio versus mixing time in CPMAS spectra of tyrosine hydrochloride.

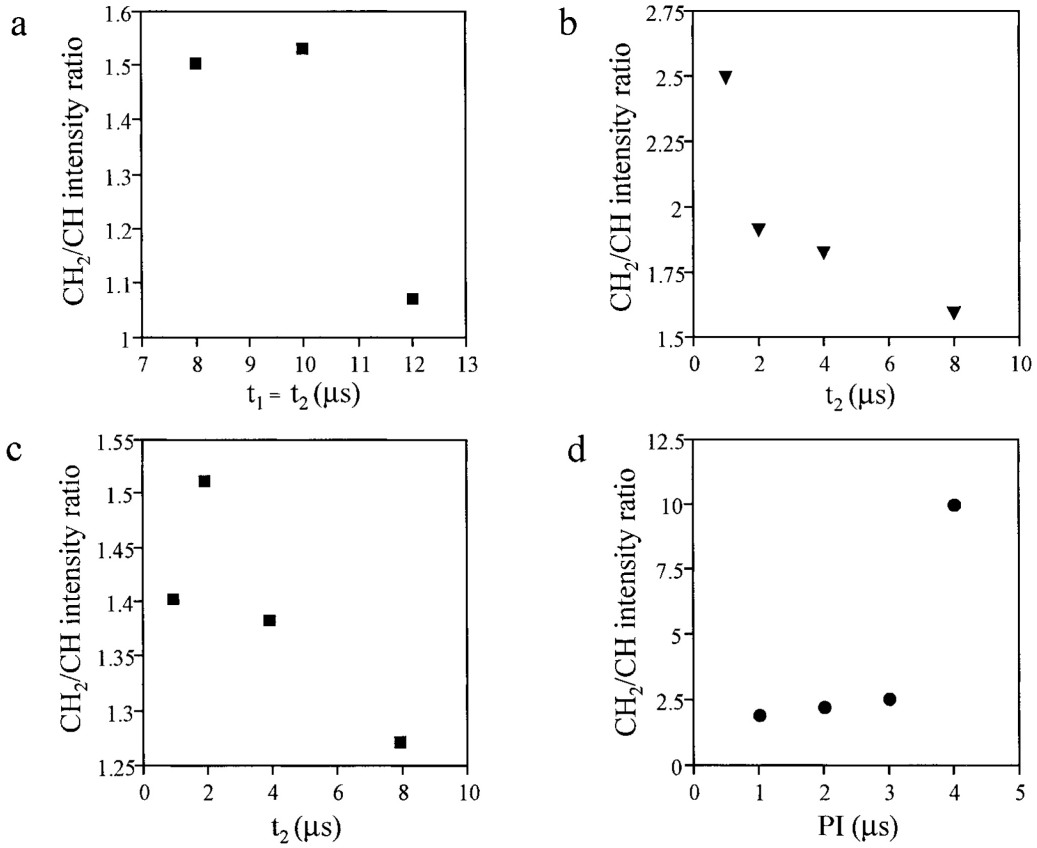

Figure 3. Optimization of DQCP(PI) sequences. The mixing time is 20 μs and the recycle delay is 4 s unless otherwise specified. Five hundred twelve acquisitions were signal-averaged for all the experiments listed. (a) Normalized CH_2/CH intensity ratio versus equal values of t_1 and t_2 with a recycle delay of 8 s. (b) Normalized CH_2/CH intensity ratio versus t_2; the t_1 value was held at 8 μs. (c) Same as (b) but with a 40 μs mixing time. (d) Normalized CH_2/CH intensity ratio versus PI. The t_1 and t_2 values are held at 8 and 1 μs, respectively. At 4 μs the CH_2/CH ratio is virtually equivalent to the S/N ratio.

The spectrum shown in Figure 4a is clearly different from the normal spectrum c but also from the DQCP spectrum b so that some question was raised on whether the spectrum was the result of DQ filtering or simply of the short CP–PI combination. In order to prove that a methylene-only spectra is by in large the result of the double-quantum filter we ran a CP–PI MAS experiment with the same 20 μs CP and 5 μs PI but no DQ filter. The results are shown in Figure5 for a sample of serine. Without DQ filtering, only the quaternary resonance is removed and most of the CH resonance is still present. By contrast, the presence of the filter produces the desired edited spectrum. Furthermore, fine-tuning of the mixing time yields inversion of the CH resonance. For a mixing time of 18 μs the CH resonance of serine appears as a low intensity artifact in the spectrum.

More samples were used to test the sequence with equally positive outcomes. Reported here are the examples of methionine and cholesteryl acetate. Methionine served to test the effects of a highly mobile methyl group on the DQ filter, and cholesteryl acetate is commonly used as a benchmark sample for editing sequences. In the methionine spectrum, the methyl is removed as well as the methyne and quaternary signals; see Figure 6. The assignment of the CH_2 peaks in cholestryl acetate

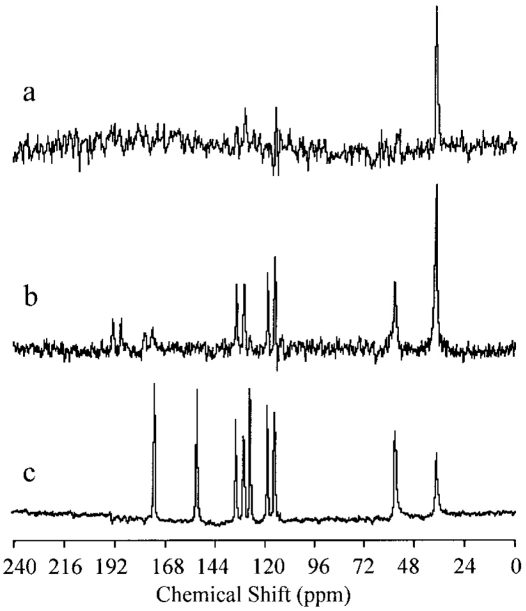

Figure 4. Comparison of DQ filtered spectra and CPMAS spectra for tyrosine hydrochloride. (a) DQCPPI with t_1 = 8 μs, t_2 = 1 μs, CP = 20 μs, PI = μs. (b) DQCP; same parameters as for (a), but no PI. (c) SELTICS spectrum (*21*). Sidebands appear in (b) in the 168–192 and 48–72 ppm regions.

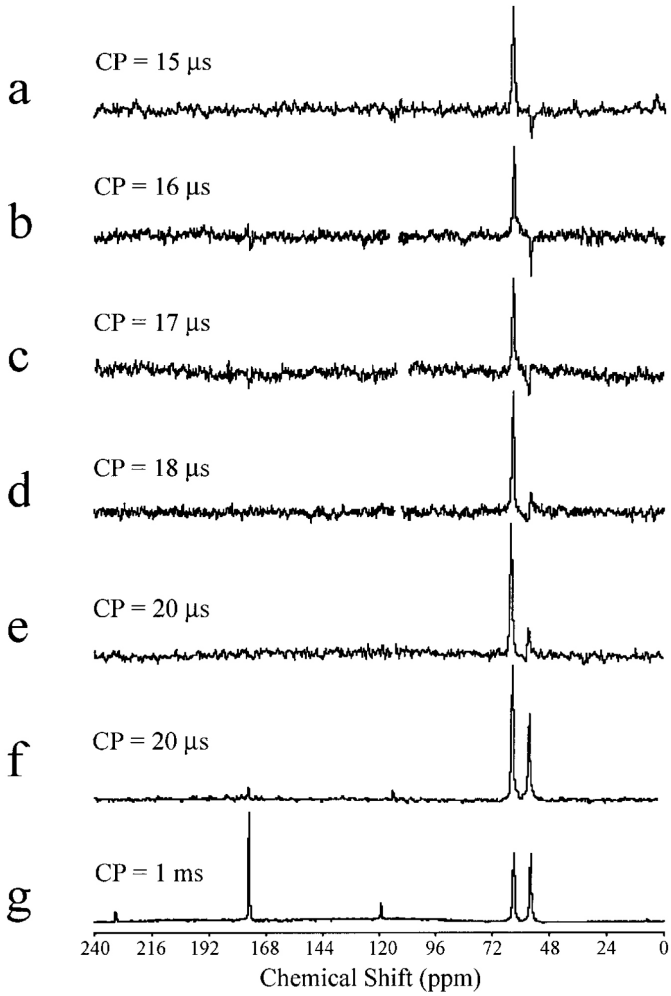

Figure 5. Mixing time optimization using a serine sample. (a)–(e) DQCPPI $t_1 = 8\ \mu s$, $t_2 = 1\ \mu s$, at fixed PI = μs. The CP time is varied as shown in the figure. (f) Short mixing CPMAS with the addition of a 5 μs PI period. (g) CPMAS spectrum, CP = 1 μs.

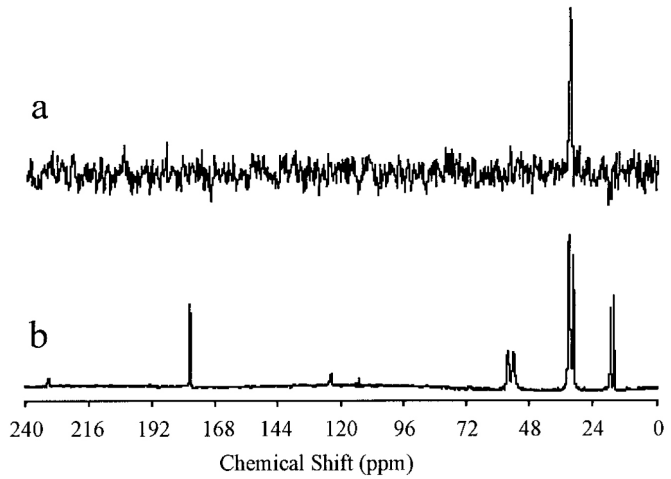

Figure 6. Methionine, edited and CPMAS spectra comparison. (a) DQCP(PI): $t_1 = 8\ \mu s$, $t_2 = 1\ \mu s$, CP = 18 μs, PI = 5 μs. (b) CPMAS: CP = 1 μs.

becomes straightforward when comparing the filtered to the unfiltered spectrum; there are a few residual peaks in the DQCPPI spectrum in Figure7 but they can be easily recognized because they are greatly attenuated with respect to the CPMAS spectrum. The most evident drawback of combined short contact time and filtering is a noisy spectrum and the need for longer acquisitions, particularly when dealing with larger systems such as cholesteryl acetate. Nonetheless, we think that our sequence is more than adequate to edit most CPMAS spectra.

We have not carried out a quantitative comparison, and the absence of experimental details makes it impossible to extract such information from their paper, but qualitatively, our editing method appears to perform as well as the Wu and Zilm sequence (*10*) in terms of signal-to-noise and residual artifacts.

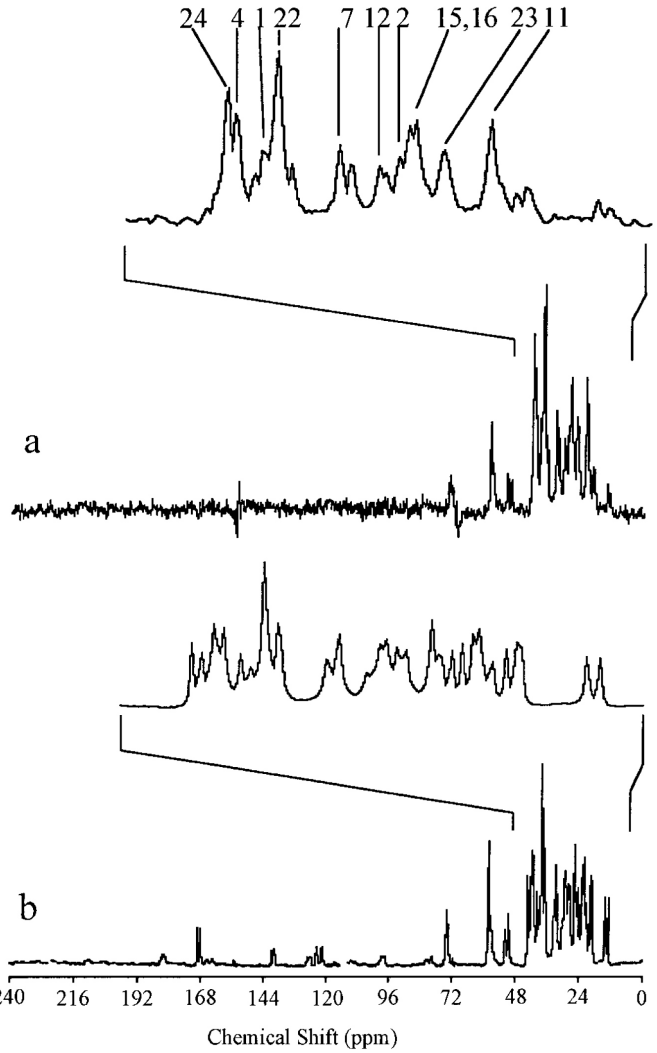

Figure 7. Cholesteryl acetete, edited and CPMAS spectra comparison. (a) DQCP(PI): $t_1 = 8\ \mu s$, $t_2 = 1\ \mu s$, CP = 18 μs, PI = 6 μs. (b) CPMAS: CP = 1 μs. The assignment of the spectrum is uses the IUPAC numbering for cholesterol.

We have described a new spectral editing technique named DQCP, double-quantum– crosspolarization, an efficient and easy to implement method to obtain methylene-only ^{13}C CPMAS subspectra of solid organic samples. The sequence has been optimized experimentally to give consistent results on a wide range of samples. Even though the requirements for the evolution time t_1 and t_2 are stringent, small adjustments to the mixing and polarization inversion times can still be made to suit the needs of each particular sample. The sequence applicability to biological samples and its further refinement is currently in progress.

Acknowledgments

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