

Methylene spectral editing in solid-state ^{13}C NMR by three-spin coherence selection

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Abstract

A robust new solid-state nuclear magnetic resonance (NMR) method for selecting CH_2 signals in magic-angle spinning (MAS) ^{13}C NMR spectra is presented. Heteronuclear dipolar evolution for a duration of 0.043 ms, under MREV-8 homonuclear proton decoupling, converts ^{13}C magnetization of CH_2 groups into two- and three-spin coherences. The CH_2 selection in the SIJ (C H H) spin system is based on the three-spin coherence $S_x I_z J_z$, which is distinguished from ^{13}C magnetization (S_x) by a ^1H $0^\circ/90^\circ$ pulse consisting of two 45° pulses. The two-spin coherences of the type $S_y I_z$ are removed by a ^{13}C 90° x -pulse. The three-spin coherence is reconverted into magnetization during the remainder of the rotation period, still under MREV-8 decoupling. The required elimination of ^{13}C chemical-shift precession is achieved by a prefocusing 180° pulse bracketed by two rotation periods. The selection of the desired three-spin coherence has an efficiency of 13% theoretically and of 8% experimentally relative to the standard CP/MAS spectrum. However, long-range couplings also produce some three-spin coherences of methine (CH) carbons. Therefore, the length of the ^{13}C pulse flipping the two-spin coherences is increased by 12% to slightly invert the CH signals arising from two-spin coherences and thus cancel the signal from long-range three-spin coherences. The signal intensity in this cleaner spectrum is 6% relative to the regular CP/TOSS spectrum. The only residual signal is from methyl groups, which are suppressed at least sixfold relative to the CH_2 peaks. The experiment is demonstrated on cholesteryl acetate and applied to two humic acids.

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1. Introduction

Spectral-editing techniques are useful tools for clear assignments of peaks in ^{13}C NMR spectra of complex organic matter and have been routinely applied in solution ^{13}C NMR. In solid-state NMR, however, these techniques are less well established. Only recently, an efficient CH selection was achieved in a “dipolar DEPT” experiment, which exploits the different dipolar-dephas-

ing properties of CH and CH_2 multiple-quantum coherences, even in the presence of partial mobility [1].

Cross-polarization (CP) inversion can select the signals of rigid CH_2 groups, at the zero-crossing of the inverted CH signal [2–4], and can be combined with ^1H double-quantum filtering before CP for improved selectivity [5]. The selected CH_2 signal in model compounds is 11–22% of the intensity in the standard CP/magic-angle spinning (CP/MAS) spectrum [2–4,6], but an application to a forest humic acid yielded only “a low-S/N spectrum” [7]. We have found the CP-based method to be highly sensitive to the Hartmann–Hahn match, requiring tedious adjustment of the cross-polarization period, with changes by $2\ \mu\text{s}$ making a significant

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difference. This is in agreement with the variety of CP times given in the literature, e.g., between 32.6 and 35 μs for the inverse-CP period [4,6,7]. It is also important to note that for systems with low sensitivity that need long signal-averaging times, the requirements on spectrometer and probehead stability are more demanding than for model compounds that can be measured with few scans.

In this paper, we present an alternative method of CH_2 spectral editing based on a completely different principle. It selects three-spin coherence that is generated within $\sim 43 \mu\text{s}$ by heteronuclear dipolar couplings in CH_2 but not CH groups. It requires homonuclear dipolar decoupling, e.g., by MREV-8 [8], but only during one rotation period t_r . It is more robust than CP methods since it does not rely on a steep zero-crossing or sensitive Hartmann–Hahn match condition.

2. Theory

2.1. Heteronuclear dipolar evolution

Fig. 1A shows the essential parts of the pulse sequence for the new CH_2 -selection technique. First we consider its effect on a CH group consisting of a S and an I -spin, with carbon magnetization S_x evolving under the heteronuclear dipolar coupling of strength $h/2\pi \omega_{\text{IS}}(t)$. Chemical-shift evolution is disregarded here but will be included below. The dipolar coupling frequency $\omega_{\text{IS}}(t)$ is time dependent due to magic-angle spinning of the sample. The density operator after a time τ , which is one MREV-8 period and equal to $t_r/4$, is [9]

$$\rho(\tau) = S_x \cos \Phi_{\text{IS}} + 2S_y I_z \sin \Phi_{\text{IS}}. \quad (1)$$

The phase Φ_{IS} is given by the integral of the instantaneous dipolar frequency

$$\Phi_{\text{IS}} = \int_0^\tau \omega_{\text{IS}}(t) dt. \quad (2)$$

The corresponding time evolution of S_x in a CH_2 group with protons I and J is easily calculated by evaluating the effects of the propagators of the commuting IS and JS interactions sequentially according to Eq. (1):

$$\begin{aligned} \rho(\tau) &= (S_x \cos \Phi_{\text{IS}} + 2S_y I_z \sin \Phi_{\text{IS}}) \cos \Phi_{\text{IS}} \\ &\quad + 2(S_y \cos \Phi_{\text{IS}} - 2S_x I_z \sin \Phi_{\text{IS}}) I_z \sin \Phi_{\text{IS}} \\ &= S_x \cos \Phi_{\text{IS}} \cos \Phi_{\text{IS}} + 2S_y I_z \sin \Phi_{\text{IS}} \cos \Phi_{\text{IS}} \\ &\quad + 2S_y I_z \sin \Phi_{\text{IS}} \cos \Phi_{\text{IS}} - 4S_x I_z \sin \Phi_{\text{IS}} \sin \Phi_{\text{IS}}. \end{aligned} \quad (3)$$

In analogy to Φ_{IS} in Eq. (2), the phase Φ_{JS} is defined in terms of the instantaneous JS dipolar-coupling frequency $\omega_{\text{JS}}(t)$

$$\Phi_{\text{JS}} = \int_0^\tau \omega_{\text{JS}}(t) dt. \quad (4)$$

Note that the z -direction of the ^1H coherences is along the effective field of MREV-8.

2.2. CH_2 selection

According to Eqs. (1) and (3), the density operators of the CH and CH_2 spin systems both contain similar single- and two-spin coherences, but the CH_2 group also produces the three-spin coherence $S_x I_z J_z \sin \Phi_{\text{IS}} \sin \Phi_{\text{JS}}$. If we can select the three-spin coherence, convert it back into observable ^{13}C magnetization and eliminate signals arising from the single- and two-spin coherences, we can select CH_2 signals. This is achieved by a ^{13}C 90° x -pulse at time τ (Fig. 1A), which makes the two-spin terms $S_y I_z$ and $S_y J_z$ unobservable, and by ^1H pulses, which discriminate between the one-spin (S_x) and three-spin ($S_x I_z J_z$) terms.

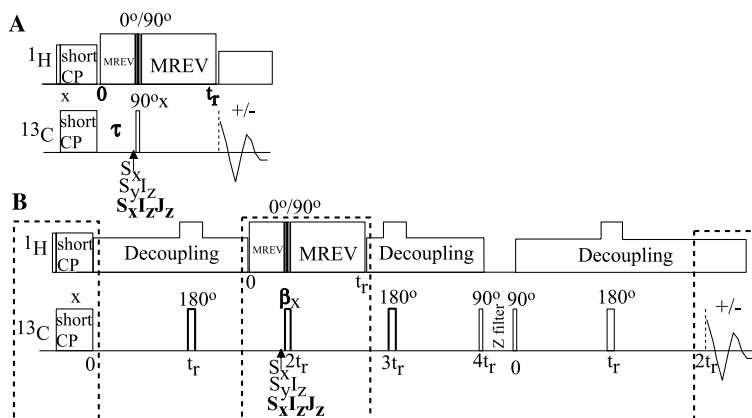


Fig. 1. Pulse sequence for CH_2 spectral editing by three-spin coherence selection. (A) Simplified version, for explaining the method while neglecting chemical-shift and long-range coupling effects. (B) Actual version with prefocusing and refocusing of ^{13}C chemical-shift precession by 180° pulses, and with a $\beta > 90^\circ$ pulse on ^{13}C to cancel signals from long-range couplings of CH groups. The basic phase cycle is indicated. The dipolar evolution time is $\tau = t_r/4$, and the total homonuclear decoupling by MREV-8 is one rotation period in both sequences. The pulse program is available from our website (<http://www.public.iastate.edu/~nmrksr/>).

More specifically, the ^{13}C 90° x -pulse is used to convert $S_y I_z$ and $S_y J_z$ into coherences that do not produce observable ^{13}C magnetization, while leaving the one- and three-spin terms unaffected. After this pulse, the spin state is

$$\rho(\tau) = S_x \cos \Phi_{1S} \cos \Phi_{JS} + 2S_z J_z \sin \Phi_{JS} \cos \Phi_{1S} \\ + 2S_z I_z \cos \Phi_{JS} \sin \Phi_{1S} - 4S_x I_z J_z \sin \Phi_{JS} \sin \Phi_{1S}. \quad (5)$$

Note that this scheme of eliminating the two-spin coherences is not sensitive to B_1 inhomogeneity. All that is required is that after the pulse, $\langle S_y I_z \rangle = 0$, where $\langle \rangle$ indicates the average over the whole sample and includes weighting by the signal-detection efficiency.

Signal from the single-spin coherence (magnetization) S_x is removed by taking the difference of scans with a 0° and with a 90° pulse on ^1H ; the two flip angles are produced by two 45° pulses with phase-alternation of the second pulse. The pulses are applied along the transverse direction perpendicular to the effective field of MREV-8 (i.e., along $\pm y$ if the effective field is along (101)). Since the ^{13}C magnetization is unaffected by the ^1H pulses, it is eliminated cleanly after two scans. Signal from the three-spin coherence is retained since the 0° pulse of the first scan leaves the density operator unchanged from Eq. (5), $\rho_0(\tau) = \rho(\tau)$, while the second scan, after the proton 90° pulse, produces

$$\rho_{90}(\tau) = S_x \cos \Phi_{1S} \cos \Phi_{JS} + 2S_z J_y \sin \Phi_{JS} \cos \Phi_{1S} \\ + 2S_z I_y \cos \Phi_{JS} \sin \Phi_{1S} - 4S_x I_y J_y \sin \Phi_{JS} \sin \Phi_{1S}. \quad (6)$$

Their difference (obtained by inverting the receiver phase for the second scan) is

$$\rho_0(\tau) - \rho_{90}(\tau) = 2(S_z I_z - S_z I_y) \cos \Phi_{JS} \sin \Phi_{1S} \\ + 2(S_z J_z - S_z J_y) \sin \Phi_{JS} \cos \Phi_{1S} \\ - 4(\underline{S_x I_z J_z} - S_x I_y J_y) \sin \Phi_{JS} \sin \Phi_{1S}. \quad (7)$$

All the coherences are subject to further evolution under the heteronuclear dipolar couplings for a period of three MREV cycles or $3/4 t_r$, which is equal to $t_r - \tau = t_r - t_r/4$ (Fig. 1A). The unobservable coherences $S_z I_z$, $S_z J_z$, and $S_x I_y J_y$ in Eq. (7) commute with $2\omega_{1S}(t) I_z S_z$ and $2\omega_{JS}(t) J_z S_z$, and therefore do not evolve under the dipolar couplings. The $S_z I_y$ and $S_z J_y$ coherences evolve into I_x and J_x , respectively, but not into S_x or S_y , and therefore do not become observable under S -spin (^{13}C) detection. Only one term in Eq. (7), the original three-spin term (underlined), converts into observable S_x magnetization, $S_x \sin^2 \Phi_{JS} \sin^2 \Phi_{1S}$, at the end of the rotation period. It has a weighting factor of $\langle \sin^2 \Phi_{JS} \sin^2 \Phi_{1S} \rangle / 2$, normalized per scan. Using $\sin^2 \Phi = (1 - \cos 2\Phi) / 2$ and $\cos \alpha \cos \beta = (\cos(\alpha + \beta) + \cos(\alpha - \beta)) / 2$, we can rewrite this weighting factor as

$$\langle \sin^2 \Phi_{JS} \sin^2 \Phi_{1S} \rangle / 2 = 1/8 \{ 1 - \langle \cos 2\Phi_{JS} \rangle - \langle \cos 2\Phi_{1S} \rangle \\ + 1/2 \langle \cos 2(\Phi_{JS} + \Phi_{1S}) \rangle \\ + 1/2 \langle \cos 2(\Phi_{JS} - \Phi_{1S}) \rangle \}. \quad (8)$$

After sufficiently long times τ compared to the inverse of the 22-kHz C–H dipolar coupling scaled by the MREV-8 scaling factor (i.e., for τ values fulfilling $2\pi \tau > 10 \text{ kHz} > \pi/2$), all the powder-averaged cosine functions approach zero, regardless of the H–C–H bond angle (as long as it is not near 0° or 180°). Thus, the theoretical signal strength (efficiency relative to a standard ^{13}C spectrum) is $1/8 = 12.5\%$.

2.3. Suppression of quaternary-C and CH_3 signals

Quaternary (i.e., unprotonated) and methyl carbons are characterized by relatively small C–H couplings. For CH_3 groups, the C–H dipolar couplings are reduced to $-1/3$ of the rigid-limit C–H couplings due to fast rotational jumps. Short CP of $100 \mu\text{s}$ suppresses these signals significantly. Furthermore, heteronuclear evolution of $\tau = 43.2 \mu\text{s}$ duration is insufficient for these weak CH couplings to generate multi-spin coherence. Indeed, we have not observed artifacts from unprotonated C, and residual CH_3 signals were seen only at an intensity level of $<1/6$ of the selected CH_2 peaks. Often, methyl signals can be further suppressed by a z-filter based on the short T_1 relaxation times of rotating CH_3 groups. Moreover, this z-filter serves to remove small out-of-phase artifacts that we could not suppress by phase cycling.

2.4. Cancellation of long-range coupling effects

So far, we have treated methine and methylene groups as isolated CH and CH_2 spin systems, respectively. In reality, however, other non-bonded protons also have dipolar couplings to the ^{13}C spin. Thus, a methine carbon is really part of a CH_n spin system and also produces three-spin coherences. Nevertheless, since all the non-bonded couplings are weak, the build-up of the methine three-spin coherences is slow. Their signal fraction relative to the selected signals of interest should be similar to the $\sim 25\%$ of quaternary carbons in the dipolar-DEPT CH-selection experiment [1]. Indeed, we observed CH signals of this magnitude in an alternative version of the pulse sequence that is quite insensitive to the pulse flip angles. It involves ^{13}C $0^\circ/180^\circ$ pulses instead of the 90° x -pulse.

These undesirable methine signals can be suppressed by increasing the ^{13}C flip angle to $\beta > 90^\circ$, as indicated in Fig. 1B. This will partially invert the $S_y I_z$ and $S_y J_z$ terms in Eq. (3), resulting in negative magnetization that cancels the long-range three-spin contributions. In our experiments, we used a $\beta = 101^\circ$ pulse. This suppressed

the CH signals, but also reduced the CH₂ signals from 8 to 6% of the corresponding CP signals.

2.5. ¹³C chemical-shift (*p*)refocusing

All the equations above did not include ¹³C chemical-shift precession, which would scramble the *S_x* and *S_y* terms and prevent the *S*-spin 90° *x*-pulse from eliminating the two-spin coherences. This chemical-shift problem is prevented by prefocusing the chemical-shift anisotropy into a Hahn echo at the time (*τ*) of the ¹³C 101° pulse in the CH₂-selection scheme, using a 180° pulse bracketed by two rotation periods, see Fig. 1B. Similarly, the chemical-shift evolution after the ¹³C 90° pulse is refocused by another 180° pulse. The ¹H coherences are along *z* and therefore not subject to chemical-shift evolution.

3. Results and discussion

3.1. Demonstration of the CH₂-selection technique on a model compound

Fig. 2 shows the application of the three-spin coherence CH₂-selection technique to cholesteryl acetate. We have chosen this model compound because it has been used as a test compound for spectral editing in the literature [1,4,5]. The molecular structure is shown in Fig. 2A. The CP/echo reference spectrum in Fig. 2B displays a large numbers of lines, many of which appear as distinct pairs, indicating that there are two chemically inequivalent molecules. Since no sideband suppression was applied, sidebands of the sp²-hybridized carbons are also observed. The CH₂-only spectrum, Fig. 2C, clearly identifies the CH₂ signals while eliminating CH and quaternary-carbon signals, and reducing CH₃ peaks to almost insignificant levels. The spectrum of Fig. 2C shows better suppression of alkyl CH signals than was achieved by CP inversion in Figs. 6 and 7d of [4], where signals of CH sites 3, 17, 20, and 8 in cholesteryl acetate are retained at a level of ca. 10%.

3.2. Technical issues

We have also tested the pulse sequence with frequency-switched Lee–Goldburg (FSLG) [10] instead of MREV decoupling at 6.5-kHz MAS. However, the efficiency with FSLG was lower than with MREV-8, probably due to the sensitivity of FSLG to B₁ inhomogeneity.

Note that no sideband suppression scheme (such as TOSS) is used in the pulse sequence of Fig. 1B. Sideband suppression is not necessary since the sidebands of most methylene signals are negligible at *ν_r* = 5787 Hz. This simplifies the experiment and avoids signal reduction due to TOSS. Exomethylenes (=CH₂), which are relatively rare,

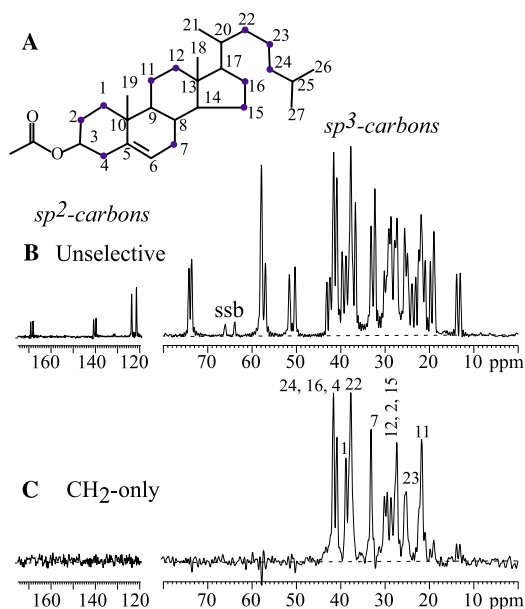


Fig. 2. CH₂ selection applied to cholesteryl acetate. (A) Molecular structure with CH₂ carbons highlighted as small filled circles. (B) Full CP/echo spectrum at *ν_r* = 5787 Hz used as a reference with 256 scans and a recycle delay of 4 s. (C) Corresponding CH₂-only spectrum at *ν_r* = 5787 Hz, with a CP time of 100 μs. A *T*_{1C} filter of 1-s duration was used to reduce the CH₃ signal intensities and to cancel small dispersive artifact signals. The number of scans was 4096, with a recycle delay of 4 s.

would produce sidebands, but their centerbands between 75 and 120 ppm [11] would be easily detectable.

The signals of mobile CH₂ groups will be reduced or suppressed, since the three-spin-selection technique relies on the evolution of the spin system under strong C–H dipolar couplings. Preliminary tests show that the new pulse sequence works well for methylene groups that are immobile enough not to retain signal after standard 40-μs dipolar dephasing. Thus, one may say that the present technique is similarly sensitive to motion as is dipolar dephasing, the most widely used spectral editing technique in solid-state NMR.

3.3. Applications to humic acids

Fig. 3 shows CP spectra of a peat humic acid and a fjord humic acid, as well as their CH₂-only spectra from three-spin coherence selection and CP inversion. With our new method, clean CH₂-only spectra are obtained from both samples (Figs. 3B and E). The spectrum of the peat humic acid (Fig. 3B) shows two distinct bands, which can be assigned to OCH₂ and CCH₂ groups, respectively. Two similar bands are observed in the CH₂-only spectrum of the fjord humic acid (Fig. 3E), but the OCH₂ signal is relatively smaller; this correlates with its smaller OCH signal, near 70 ppm, which suggests that OCH and OCH₂ groups are predominantly due to sugar rings.

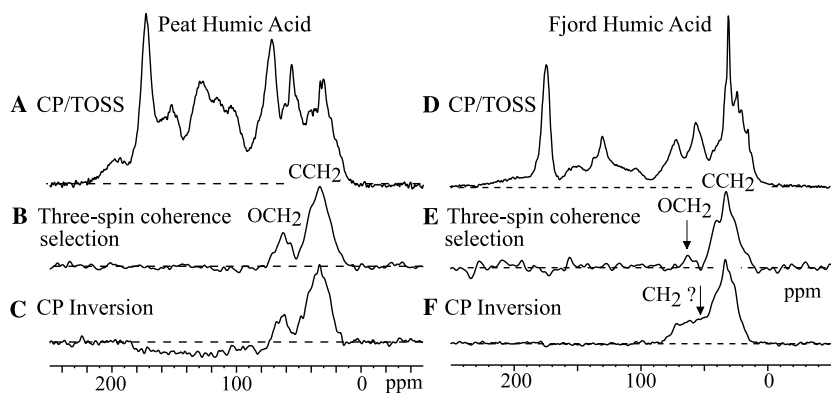


Fig. 3. CH_2 selection applied to a peat humic acid and a fjord humic acid. Spectra (A–C) are of the peat humic acid, (D–F) of the fjord humic acid. (A and D) full CP/TOSS spectra for reference, acquired at $\nu_r = 5$ kHz with a CP time of 1 ms. (B and E) CH_2 -only spectra obtained using three-spin coherence selection at $\nu_r = 5787$ Hz, with a CP time of 100 μs . A $T_{1,C}$ filter of 0.1-s duration was used to reduce the CH_3 signal intensities and to cancel potential dispersive artefact signals. (C and F) CH_2 -only spectra obtained using the CP-inversion technique at $\nu_r = 5$ kHz. The initial CP time was 40 μs , the inversion LGCP time 52 and 49 μs for (C and F), respectively. Total measuring times: (A) 0.28 h, (B) 10.2 h, (C) 0.68 h, (D) 1.7 h, (E) 16.4 h, and (F) 3.4 h.

The corresponding spectra obtained using the CP-inversion method demonstrate limitations of the CP inversion technique (Figs. 3C and F). The CP-inversion CH_2 -only spectrum of peat humic acid (Fig. 3C) shows negative peaks at >70 ppm, underlining that this technique relies sensitively on the zero-crossing of the inverted polarization. Similar negative signals are also observed in the CP-inversion “ CH_2 -only” spectrum of a forest humic acid, shown in Fig. 5c of [7]. If the CP-inversion time is not chosen very accurately, such residual positive or negative CH signals will be obtained. This problem is especially serious for complicated natural organic matter because of heavily overlapped lines. In practice, we have had to use slightly different CP times to obtain “ CH_2 -only” spectra of various samples. This sometimes works but other times fails as small artifact signals are revealed after long signal averaging, possibly due to spectrometer power-level drift. The CP-inversion CH_2 -only spectrum of fjord humic acid (Fig. 3F) apparently shows residual NCH signals, while the corresponding CH_2 -only spectrum obtained using the three-spin coherence technique displays clean CH_2 selection (Fig. 3E). Thus the three-spin coherence technique is more reliable, eliminating the need for tedious adjustment of CP times or power levels and removing the sensitivity to pulse power-level fluctuations.

3.4. Outlook for three-spin coherence selection under fast(er) MAS

It should be relatively straightforward to adapt the three-spin coherence selection to higher spinning frequencies. For instance, when spinning at 10 kHz, the selection pulses in Fig. 1B would be applied at $\tau = 50 \mu\text{s} = t_r/2$, with homonuclear decoupling by FSLG. When spinning at 20 kHz, heteronuclear excitation should again take $\tau = 50 \mu\text{s}$. Since this is now a full

rotation period, a ^{13}C 180° pulse at $t_r/2$ is required for recoupling. After the selection pulses on ^{13}C and ^1H , reconversion of the three-spin coherence into magnetization will occur during a second rotation period, again with a ^{13}C 180° pulse for recoupling. In this case, additional prefocusing and refocusing ^{13}C 180° pulses are not necessary.

4. Conclusions

A robust new solid-state NMR method for selecting CH_2 ^{13}C signals has been introduced. This technique selects the three-spin coherence in the CH_2 system after 43 μs of heteronuclear evolution while eliminating one-spin and two-spin coherences. It avoids problems of the CP-inversion technique such as reliance on tedious adjustment of CP to achieve an exact zero-crossing of all the CH signals. The CH_2 signal intensity after three-spin coherence selection is ca. 6% relative to the regular CP/TOSS spectrum. Only one rotation period of homonuclear decoupling, by MREV-8, is required. By using short CP and short heteronuclear dipolar evolution times, the quaternary carbons are fully suppressed, while the methyl signals are reduced to an intensity of $<1/6$ relative to that of CH_2 peaks. The new method provides reliable CH_2 selection on natural organic matter within acceptable measuring times.

5. Experimental

5.1. Samples

Cholesteryl acetate was purchased from Sigma–Aldrich. A peat humic acid was extracted from a peat in Amherst, HA [1,12]; a fjord humic acid (F-20-HA)

was kindly provided by Dr. Luc Tremblay and Dr. Jean-Pierre Gagne from the Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, Que., Canada. The extraction and detailed characterization of these base-soluble organic fraction have been described elsewhere [12,13].

5.2. NMR parameters

Experiments were performed using a Bruker DSX400 spectrometer at 100 MHz for ^{13}C , with a Bruker 7-mm magic-angle spinning double resonance probehead. For the three-spin coherence experiments, the ^{13}C 90° pulse length was 3.9 μs . For the proton homonuclear decoupling, the radio frequency power was relatively high, corresponding to a $\sim 3.4\text{-}\mu\text{s}$ 90° pulse and a 43.2- μs cycle time of the semi-windowless MREV-8 decoupling sequence; the heteronuclear evolution time $\tau = 43.2\text{ }\mu\text{s}$ is 1/4 of the rotation period at the spinning frequency of 5787 Hz. The CP contact time was 100 μs . No tune-up of MREV-8, other than setting of the nominal 90° pulse, was performed.

For the CP-inversion experiments, the first CP contact time was 40 μs and the second contact time, with Lee–Goldburg CP inversion, was 52 μs for the peat humic acid and 49 μs for the fjord humic acid. The spinning speed was 5 kHz.

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