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# Modified Spectral Editing Methods for $^{13}C$ CP/MAS Experiments in Solids

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**The spectral editing approach of Zilm and coworkers utilizes polarization, polarization inversion, and spin depolarization methods for enhancing or suppressing NMR spectral lines in solids. The proposed pulse sequences allow nonprotonated C, CH, $CH_2$, and $CH_3$ types of carbon resonances to be separated from one another and identified accordingly. The former method tentatively separates the nonprotonated C and $CH_3$ peaks with a cutoff shift of 35 ppm. This shift is a reasonable demarcation shift for a preponderance of organic molecules, but exceptions do exist that could constitute a serious drawback in a few instances. The new approach separates the nonprotonated C and $CH_3$ carbon peaks unequivocally using modified pulse sequences similar to those of Zilm. Further, both the CH only and $CH_2$ only spectra, respectively, can be acquired directly from combining so called (+) and (−) sequences using different spectral delay periods and pulse parameters. The (+) and the (−) pulse sequences produce signals for the nonprotonated and methyl carbons that have essentially the same amplitude but opposite phases. These spectra, combined with the previously reported $CH_3$ and nonprontonated C only spectra, offer a complete spectral editing technique for solid samples. Examples of these spectral editing methods are provided for 3-methylglutaric acid, fumaric acid monoethyl ester, and two complex natural products: methyl *o*-methylpodocarpate and 10-deacetylbaccatin III.** © 2000 Academic Press

*Key Words:* **$^{13}C$ CP/MAS; spectral editing.**

## I. INTRODUCTION

In the past two decades, successful editing techniques have been developed for interpreting solid CP/MAS (cross polarization/magic angle spinning) NMR spectra (*1–8*). The recent appealing approach proposed by Wu *et al.* (*7*) provides both practical and convenient spectral editing methods for chemists. These workers identified a suite of five different editing experiments. They are (a) the standard CP/MAS spectrum with a long cross polarization (LCP) time. (b) A short cross polarization (SCP) CP/MAS spectrum favors CH and $CH_2$ peaks while suppressing the nonprotonated C and $CH_3$ peaks. (c) A so-called SCPPI $CH_2$ only spectrum is obtained from a SCP spectrum combined with synchronous phase inversion (PI). (d) A SCPD combined nonprotonated C and $CH_3$ spectrum is obtained from the SCP experiment followed by depolarization (D). Finally, (e) a LCP spectrum with depolarization (LCPD) also yields the nonprotonated C and $CH_3$ peaks but with greater signal to noise ratio (S/N).

A designated cutoff frequency of 35 ppm is used to separate the nonprotonated peak (>35 ppm) from the methyl peak (<35 ppm) appearing in both experiments (d) and (e). Zilm *et al.* (*7*) acknowledge that this presumption is the "principal weakness" (though not fatal) of their approach. For example, methyls directly bonded to electronegative atoms such as oxygen and nitrogen may have shifts > 35 ppm. Finally, a residual CH only spectrum is synthesized by combining the various experimental spectra with appropriate weighting coefficients to null the other peaks. It would be desirable if a CH only spectrum could be acquired directly instead of by difference between other spectra. This manuscript suggests remedies for these two shortcomings in the spectral editing methods.

## II. EXPERIMENTAL

### 1. Compounds

3-Methylglutaric acid [MGA] (99%), fumaric acid monoethyl ester [FAME] (95%), and methyl *o*-methylpodocarpate [1231-74-9] (97%) were obtained from Aldrich and used as received. The 10-deacetylbaccatin III sample was obtained from Bristol Meyers Squibb and prepared by crystallizing from dimethyl sulfoxide (DMSO).

### 2. Instrumentation

All the spectra were acquired on a CMX 200 NMR spectrometer with $^{13}C$ and $^{1}H$ Larmor frequencies of 50.308 and 200.04 MHz, respectively. The commercial 7.5-mm CP/MAS probe with a speed controller came from Chemagnetics. A proton RF field of 62.5 KHz gave a $\pi/2$ pulse width of 4 $\mu$s. The sample spinning rate was 4 KHz.

### 3. Pulse Sequence and Spectroscopic Parameters

The new pulse sequences given in Fig. 1 for spectral editing follow the notations used in the original paper (*7*). Typical



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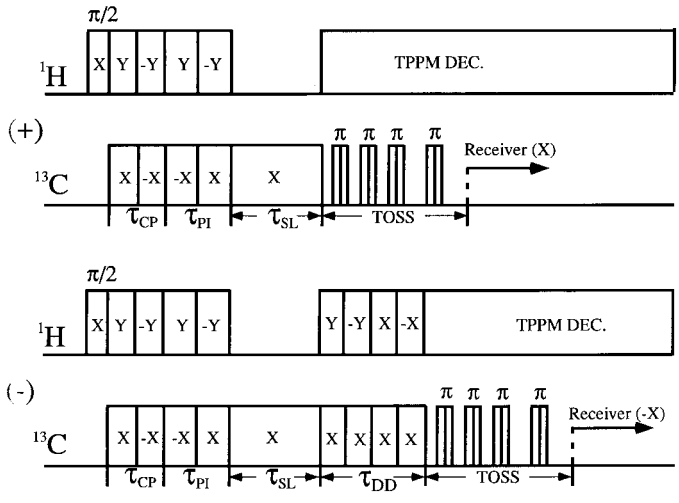


**FIG. 1.** The pulse sequences used for spectral editing in $^{13}$C CP/MAS experiment.

parameters that produce each subspectrum are summarized in Table 1. These representative parameters were also found for several similar samples. Minor improvements between the current and former pulse sequences include, (1) the TPPM (*9*) used to enhance proton decoupling and (2) a conventional 4-$\pi$ TOSS pulse sequence (*10*). In our implementation, the TPPM cycle consisted of two $\pi$ pulses with a 16° phase difference between them. The TOSS sequence was used to eliminate the spinning sidebands associated with chemical shift anisotropy and the combination of TOSS with the original spectral editing techniques had been demonstrated previously (*11*). In summary, the experiments for the revised spectral editing include:

*a. The standard $^{13}$C CP/MAS spectrum (all carbons).* The conventional CP/MAS spectrum is acquired in the same way as reported in the original paper (*7*) using the (+) pulse sequence in Fig. 1. The parameter settings are $\tau_{CP}$ = 1–8 ms, depending upon the $^1$H $T_{1\rho}$ of the system investigated; the polarization inversion time is $\tau_{PI}$ = 0.1 $\mu$s. The relative sensitivities of the other spectra are then compared with this experiment normalized to 100% on a per unit time basis.

*b. Nonprotonated C and $CH_3$ only spectrum (C + $CH_3$).* This spectrum is generated in accordance with the original prescriptions (*7*) using the (−) pulse sequence in Fig. 1. Comparative sensitivities are 75–85% for nonprotonated carbons and about 50% for methyl carbons.

*c. Nonprotonated only (C) spectrum.* The nonprotonated only spectrum is also obtained using the (−) pulse sequence in Fig. 1. Both the nonprotonated carbons and the methyl carbons are polarized appreciably with a contact time ($\tau_{CP}$) of 400 $\mu$s. When this is followed by a polarization inversion time of $\tau_{PI}$ = 141 $\mu$s, the signal from the methyl carbons is nulled. Signal from the CH and $CH_2$ carbons are further suppressed through depolarization with $\tau_{DD}$ = 100 $\mu$s, leaving only nonprotonated C signals during data acquisition. The comparative sensitivity is 10–17% depending upon the magnitude of the $^{13}$C–$^1$H dipolar interaction. This removes any ambiguity between nonprotonated C and $CH_3$ peaks that may exist in Experiment (b).

*d. CH only spectrum.* $CH_2$ and CH spectral peaks may be generated using the (+) sequence with a SCP time of $\tau_{CP}$ = 40 $\mu$s, and they polarize to about $\frac{2}{3}$ and $\frac{1}{2}$ of their full intensities, respectively. The nonprotonated C and $CH_3$ carbons, though small compared to both CH and $CH_2$ carbons, are still present as partially polarized peaks. A polarization inversion pulse $\tau_{PI}$ of about 25.3 $\mu$s will then null the $CH_2$ carbon signals. This produces a FID whose FT spectrum contains a positive CH signal plus smaller positive signals from both nonprotonated C and $CH_3$ carbons. The signal from nonprotonated C and $CH_3$ signals are canceled by adding the FID from the (−) pulse sequence with a short contact time of $\tau_{CP}$ = 34.6 $\mu$s, with $\tau_{PI}$ = 0.1 $\mu$s and $\tau_{DD}$ = 100 $\mu$s. The purpose of the (−) pulse sequence is to generate a FT spectrum that contains only nonprotonated C and $CH_3$ signals with essentially the same amplitude but negative phase relative to their counterparts from the (+) sequence. By alternatively sampling the FID from the (+) and the (−) pulse sequence and combining them, a CH only spectrum is generated. The residual signals from the nonprotonated and the methyl carbons may be minimized by a fine adjustments of the $\tau_{CP}$ parameter in the (−) pulse sequence. The relative sensitivity is about 9%.

*e. $CH_2$ only spectrum.* A spectrum containing only $CH_2$ signals may be acquired in a manner similar to the CH only spectrum. The CH signal is nulled in the (+) sequence by setting $\tau_{CP}$ = 80 $\mu$s followed by $\tau_{PI}$ = 32.6 $\mu$s. This results in a FID whose frequency spectrum contains only $CH_2$ signals with negative phase and nonprotonated and $CH_3$ signals with positive phase. The nonprotonated and $CH_3$ signals are then eliminated again by alternatively adding the FID acquired using the (−) pulse sequence with $\tau_{CP}$ = 57.5 $\mu$s, $\tau_{PI}$ = 0.1 $\mu$s and $\tau_{DD}$ = 100 $\mu$s. The (−) pulse sequence generates a FID whose FT spectrum contains only nonprotonated C and $CH_3$ signals with essentially the same amplitude but negative phase relative to their counterparts produced by the corresponding (+) sequence. Relative sensitivity is about 11%.

The above protocol for spectral editing has been used for molecules that serve as calibration standards, viz. 3-methylglu-

**TABLE 1**
**Parameters Used in Fig. 1 to Generate $^{13}$C Subspectra**

| Subspectra | Pulse sequence | $\tau_{CP}$ ($\mu$s) | $\tau_{PI}$ ($\mu$s) | $\tau_{DD}$ ($\mu$s) | $\tau_{SL}$ ($\mu$s) |
|---|---|---|---|---|---|
| All | (+) | 4000 | 0.1 | — | 200 |
| C+$CH_3$ | (−) | 4000 | 0.1 | 100 | 100 |
| C | (−) | 400 | 141 | 100 | 100 |
| CH | (+) | 40 | 25.3 | — | 200 |
| | (−) | 34.6 | 0.1 | 100 | 100 |
| $CH_2$ | (+) | 80 | 32.6 | — | 200 |
| | (−) | 57.5 | 0.1 | 100 | 100 |

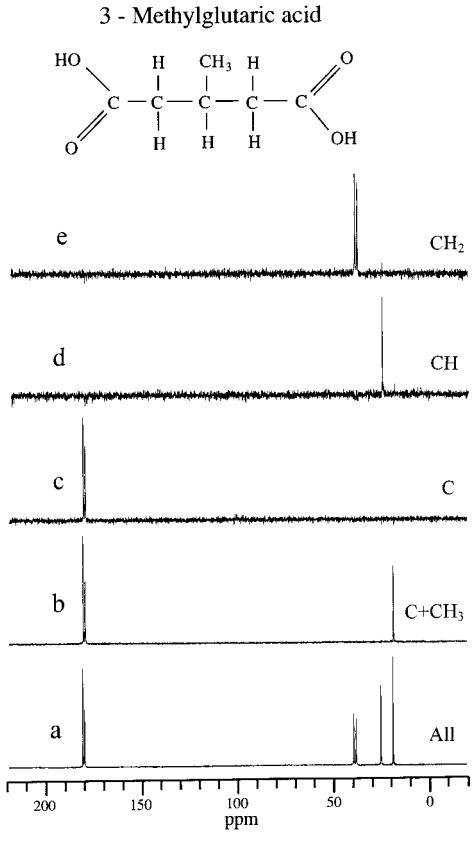


**FIG. 2.** The five experimental spectra of 3-methylglutaric acid (~250 mg). The accumulation numbers are (a) 32, (b) 24, (c) 148, (d) 52 for both the (+) and the (−) sequences, and (e) 92 for both the (+) and the (−) sequences.

taric acid and monoethyl fumaric acid ester. Since the spectroscopic parameters are sensitive to the CP match condition between the two RF channels, the spectroscopist is required to establish his own empirical parameters before proceeding with spectral editing on samples with unknown structures.

In the present experiments, the spectrometer was set up using the following procedures. The cross-polarization matching condition at 4 kHz was identified using adamantane. The optimum TPPM decoupler frequency was determined using MGA by arraying the decoupler carrier frequency to find the optimum decoupler frequency which produces the best resolution for the $CH_2$ carbon. The CP match condition under the chosen decoupler offset is then tuned again using adamantane.

## III. RESULTS AND DISCUSSION

The five experimental spectra for both MGA and FAME are presented in Figs. 2 and 3, respectively. The particular success of the protocol given in this paper is in the nonprotonated only spectra (Figs. 2c and 3c), and CH only subspectra (Figs. 2d and 3d). Compared to the standard CP experiment (Figs. 2a and 3a), the relative intensities for the two $CH_2$ carbons in Fig. 2e and the relative intensities for the two CH carbons in Fig. 3d are well preserved. However, appreciable differences in nonprotonated C intensities (Figs. 2c and 3c) are noted. These differences, due to variations in remote proton dipolar couplings, are manifest in the spectral intensities because of long polarization inversion times (141 $\mu$s) required to null the methyl signals. Consequently, the nonprotonated C only intensities, while only semiquantitative, are sufficient for the purpose of spectral identification. The difference in long-range proton–carbon dipolar coupling for nonprotonated carbons is barely recognized in the C + $CH_3$ only spectrum (Fig. 3b), whereas the intensity differences in Fig. 3c are more pronounced.

A careful comparison made between the $CH_2$ only spectrum acquired using the present strategy with that acquired using SCPPI by Wu *et al.* (*7*) suggested that a high quality $CH_2$ only spectrum was somewhat easier to obtain with this new method. This result was due to the very high quality of the high power transmitter and the stability of the probe required to null the nonprotonated and the methyl carbons in the SCPPI sequence. When the transmitter and the probe changed slightly, a precise

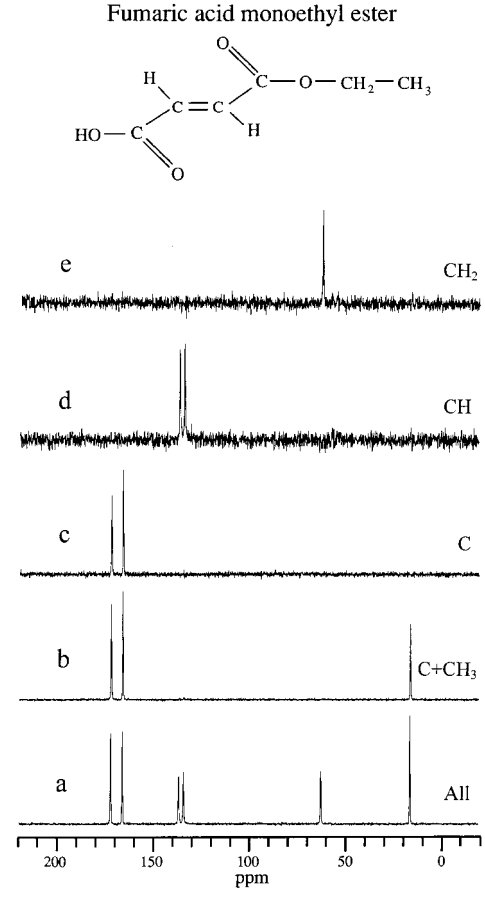


**FIG. 3.** The five experimental spectra of fumaric acid monoethyl ester (~250 mg). The accumulation numbers are (a) 32, (b) 64, (c) 256, (d) 164 for both the (+) and the (−) sequences, and (e) 140 for both the (+) and the (−) sequences.

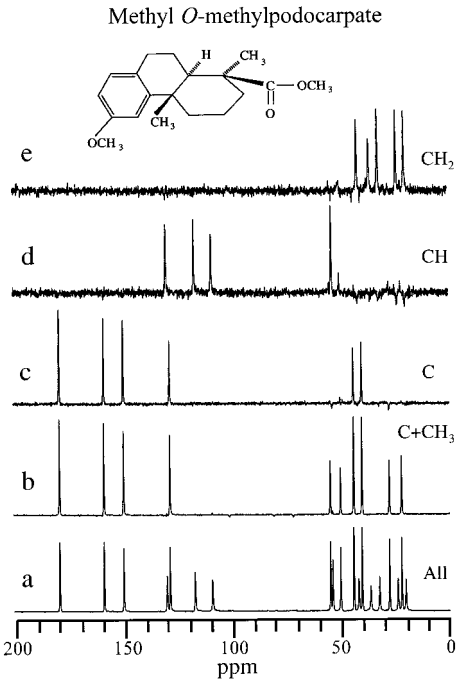


**FIG. 4.** The five experimental spectra of methyl *o*-methylpodocarpate (~250 mg). The accumulation numbers are (a) 1000, (b) 1000, (c) 3000, (d) 1500 for both the (+) and the (−) sequences, and (e) 1500 for both the (+) and the (−) sequences.

match was difficult to maintain, and signals from both nonprotonated and methyl carbons became more noticeable. In the current approach, the combined (+) and the (−) pulse sequences are more forgiving in canceling some of these effects and it is easier to get a $CH_2$ only spectrum. The drawback of this otherwise favorable situation is a reduction in the overall S/N of the new method compared with that of the SCPPI experiment (*7*).

The respective experimental parameters used to acquire the spectra in Figs. 2 and 3 are very similar, indicating that considerable generality exists in spectral editing. To illustrate the approach, the method is applied in methyl *o*-methylpodocarpate where the chemical shift ranges of methyl and nonprotonated C overlap and no cut-off shift can apply. The 10-deacetylbaccatin III spectrum offers a different challenge as all of the $CH_2$ resonances are heavily overlapped with each other and with other types of carbons.

The five experimental spectra of methyl *o*-methylpodocarpate are given in Fig. 4. In the conventional CP spectrum in Fig. 4a, there are 19 well-resolved resonances corresponding to the 19 carbons in this molecule. It is clear from Fig. 4c that the chemical shift values for the 6 nonprotonated carbons are identified as 40.1, 43.9, 128.4, 149.7, 158.7, and 179 ppm, respectively. Comparing Fig. 4b with 4c, the chemical shift values for the 4 methyl carbons are identified and they appear at 22.0, 27.6, 49.9, and 54.6 ppm, respectively. The chemical shift values for the 4 CH carbons (53.5, 108.9, 116.9, and 129.8 ppm) and the 5 $CH_2$ carbons (20.2, 23.8, 32.2, 36.3, and 41.7 ppm) are directly obtained from Figs. 4d and 4e, respectively.

The five experimental spectra for 10-deacetylbaccatin III are found in Fig. 5. The complete assignments will be published elsewhere (*12a*). It is known from Fig. 5 that a $CH_2$ resonance ($C_{20}$) is degenerate with a nonprotonated resonance ($C_1$) at 78.8 ppm. Also, the two $CH_2$ resonances at 37.7 ($C_6$) and 41.3 ppm ($C_{14}$) are superimposed with the broad methyl resonance (39.6 ppm) from the inclusion of DMSO which was used as a solvent in crystallizing the sample. The ability to identify these $CH_2$ carbon resonances [$C_6$, $C_{14}$, and $C_{20}$] in the presence of both broadened and overlapping peaks demonstrates the power of these spectral editing techniques. It is observed that the methyl resonance from the included DMSO appears in the nonprotonated only spectrum in Fig. 5c. This abnormality arises because one of the two methyls from the inclusion of DMSO molecule is thermally disordered (*12b*), i.e., undergoing some kind of isotropic tumbling. Motion makes the methyl carbon in DMSO indistinguishable from the nonprotonated C in 10-deacetylbaccatin III based on the H–C dipolar interaction.

The distinctively different rates of polarization, polarization

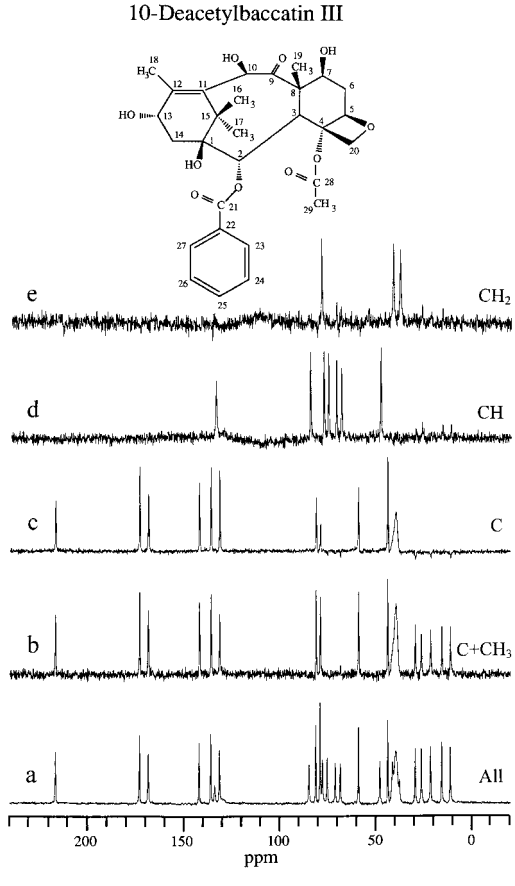


**FIG. 5.** The five experimental spectra of 10-deacetylbaccatin III(~130 mg). The accumulation numbers are (a) 2168, (b) 200, (c) 14088, (d) 10428 for both the (+) and the (−) sequences, and (e) 8304 for both the (+) and the (−) sequences.

inversion, and depolarization for various functional groups, which are determined by the number of proton attachments and the effective dipolar interactions between $^{13}C$ and $^{1}H$, are the basis of the spectral editing reported in this work. In rigid polycrystalline samples, the C–H bond lengths in CH, $CH_2$, and $CH_3$ groups are essentially the same, resulting in a similar C–H dipolar coupling constant for each type of functional group. As a result, a consistent set of experimental parameters exists that may be used in identifying CH and $CH_2$ groups for spectral editing. At room temperature, the $CH_3$ group normally rotates about its $C_3$ symmetric axis at a rate that exceeds the static dipolar $^{13}C$–$^{1}H$ dipolar coupling, thereby reducing the dipolar coupling in a $CH_3$ group to 1/3 of its static value. Consequently, a consistent polarization inversion time may be employed to null the $CH_3$ groups in a polycrystalline sample and leave a nonprotonated only spectrum. The experimental parameters need to be modified slightly to generate clean subspectra when significant variations in remote proton–carbon dipolar couplings are encountered. Fortunately, the modification of the parameters is usually modest and readily determined.

When molecular motion is involved, the dipolar interaction is suppressed as noted for the inclusion peak from DMSO solvent used in crystallizing 10-deacetylbaccatin III. Similarly, the method may fail when an inordinate high spinning rate in the MAS experiment is used that substantially suppresses the dipolar interaction. In these cases, a method reported recently by Lesage *et al.* (*8*) that was developed based on the scalar carbon–proton interaction, may be considered as complementary to the spectral editing techniques reported in this work.

## IV. CONCLUSION

This work demonstrates that a nonprotonated C only spectrum free of $CH_3$ peaks may be readily acquired, eliminating the assumption that the nonprotonated C and the $CH_3$ carbons must be separated into distinct chemical shift ranges. The CH only spectrum was determined directly using both the (+) and the (−) pulse sequences in an interleaved manner. By adding alternatively acquired FIDs together on a short time scale, long time instability does not influence the combination of successive FIDs even when the instrument undergoes minor drifts in its settings. This cancellation of errors allows the new modifications to generate CH only spectrum without undue propagation of errors. The $CH_2$ only spectrum in this work is obtained directly in a manner similar to the CH only spectrum using different delays and pulse parameters. These new methods are comparable to Zilm's methods for $CH_2$ only spectra. In spite of the benefits, however, this convenient approach does suffer from a lower S/N compared to the previous SCPPI method used to obtain a $CH_2$ only spectrum.

## ACKNOWLEDGMENTS

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