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Note

CH$_n$ Subspectral Editing with a Modified CAPT Sequence

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ABSTRACT: A modified version of the CAPT sequence with appropriate phase cycling is shown to give $^{13}C\{^1H\}$ subspectra containing only quaternary carbon resonances; only methylene resonances; only methyl resonances; methine and methyl resonances; or quaternary and methylene carbon resonances. Subtraction of the methyl-only spectrum from the methine and methyl spectrum produces a subspectrum containing only the methine carbon resonances. © 1998 John Wiley & Sons Ltd.

KEYWORDS: APT; NMR; ^{13}C NMR; spectral editing

INTRODUCTION

A variety of pulse sequences, including APT,[1] CAPT,[2] INEPT,[3] DEPT,[4] spin-flip,[5] SEMUT[6] and PENDANT,[7] allow straightforward recognition of ^{13}C resonances due to quaternary, methine, methylene and methyl carbons. In the APT experiment (a commonly used sequence for multiplicity identification) with a spin-echo delay time of 0.008 s, resonances from quaternary and methylene carbons are 180° out of phase with respect to those from methine and methyl carbons. Spectral editing to obtain subspectra containing only quaternary and methylene ('C and CH$_2$') or methine and methyl ('CH and CH$_3$') carbon resonances may be generated by addition or subtraction of the FID obtained with the proton decoupler gated off and the FID obtained with the decoupler left on during the spin-echo delay. Further editing may be realized with three other values of the spin-echo delay. A subspectrum with resonances from only quaternary carbons is generated using a delay of 0.004 and a methine subspectrum is produced by subtraction of the FIDs obtained with delays of 0.0032 and 0.0048 s.[1] In this paper, we show how the CAPT pulse sequence[2] can be used to create heteronuclear multiple quantum coherence and, with appropriate phase cycling, generate subspectra containing resonances from only quaternary carbons ('C only'), C and CH$_2$ carbons, CH and CH$_3$ carbons, only methylene carbons ('CH$_2$ only') or only methyl carbons ('CH$_3$ only') without the need for addition or subtraction of FIDs.

RESULTS AND DISCUSSION

The CAPT pulse sequence[2] shown in Fig. 1 was developed as a '*J*-compensated' version of APT which gives effective multiplicity identification over a much broader range of values of the one-bond carbon–proton coupling constant, J^1_{CH}, than APT. Also shown in Fig. 1 are the coherence pathways followed by the magnetizations for quaternary, methine, methylene and methyl carbons. For clarity, a brief description of the behavior of the various spin systems as they progress through the CAPT sequence is given here in order to show how subspectral editing is obtained. The first ^{13}C pulse creates transverse carbon magnetization (product operator C_{-1}) which is allowed to couple with its proton partner(s) during the first τ period (chemical shift effects can be ignored throughout since they are refocused by

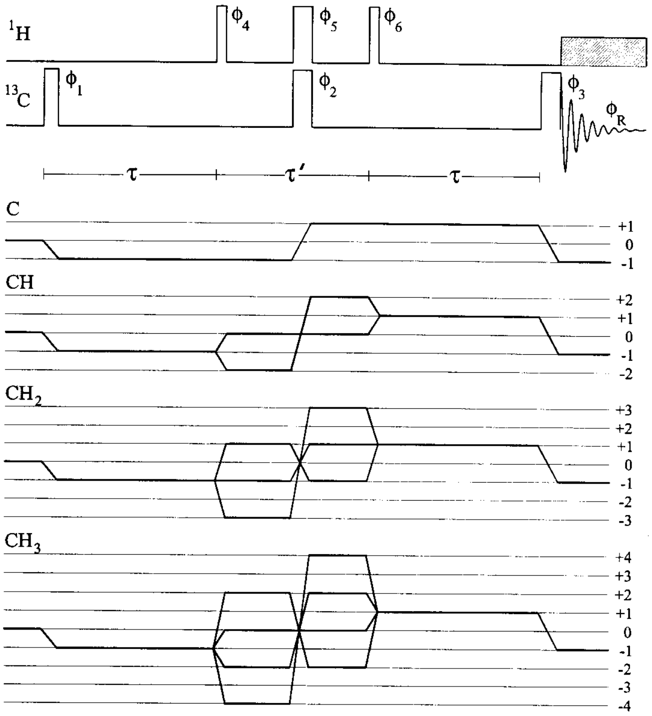

Figure 1. CAPT pulse sequence and coherence transfer pathways for quaternary (C), methine (CH), methylene (CH$_2$) and methyl (CH$_3$) spin systems.

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the ^{13}C 180° pulse). At the end of this delay, the important coherences for C, CH, CH_2 and CH_3 spin systems are those associated with the product operators C_{-1}, $2H_0^{(1)}C_{-1}$, $4H_0^{(1)}H_0^{(2)}C_{-1}$ and $8H_0^{(1)}H_0^{(2)}H_0^{(3)}C_{-1}$, respectively. Here we use the symbols C and H to represent the angular momentum operators for the carbon and proton spins, and use spherical components[8] because each product operator is unambiguously associated with a single coherence level. The C_{-1} coherence from the quaternary carbons is unaffected by all ^{1}H pulses, but the first 90°_H pulse transfers the coherences from level $p = -1$ into heteronuclear multiple quantum coherences for the CH_n spin systems: $2H_{\pm 1}^{(1)}C_{-1}$ ($p = 0, -2$) for CH; $4H_{\pm 1}^{(1)}H_{\pm 1}^{(2)}C_{-1}$ ($p = +1, -3$) and $4H_{\pm 1}^{(1)}H_{\mp 1}^{(2)}C_{-1}$ ($p = -1$) for CH_2; and $8H_{\pm 1}^{(1)}H_{\pm 1}^{(2)}H_{\pm 1}^{(3)}C_{-1}$ ($p = +2, -4$) and $8H_{\pm 1}^{(1)}H_{\pm 1}^{(2)}H_{\mp 1}^{(3)}C_{-1}$ ($p = 0, -2$) for CH_3. All of these coherences are invariant to spin–spin coupling and chemical shift precession during the $\tau'/2\text{–}180^\circ_{H,C}\text{–}\tau'/2$ period so that the $180^\circ_{H,C}$ pulses simply invert the signs of the spherical components of all operators, and the second ^{1}H 90° pulse converts the multiple quantum coherences into coherence level + 1 antiphase carbon magnetizations. The carbon magnetizations (C_{+1}, $2H_0^{(1)}C_{+1}$, $4H_0^{(1)}H_0^{(2)}C_{+1}$ and $8H_0^{(1)}H_0^{(2)}H_0^{(3)}C_{+1}$ for C, CH, CH_2 and CH_3, respectively) have phase differences which reflect the phases of the ^{1}H pulses and the spin system: 0 for C; $\pm(\phi_4 - 2\phi_5 + \phi_6)$ for CH; 0 and $\pm 2(\phi_4 - 2\phi_5 + \phi_6)$ for CH_2; and $\pm(\phi_4 - 2\phi_5 + \phi_6)$ and $\pm 3(\phi_4 - 2\phi_5 + \phi_6)$ for CH_3. In the original CAPT implementation, the phases of the proton pulses were chosen so that the antiphase carbon magnetizations were effectively 'stored' as multiple quantum coherences during the $\tau'/2\text{–}180^\circ_{H,C}\text{–}\tau'/2$ period and were simply inverted in sign and carbon coherence level by the $90^\circ_H\text{–}\tau'/2\text{–}180^\circ_{H,C}\text{–}\tau'/2\text{–}90^\circ_H$ part of the sequence, and the value of τ' was chosen in order to maximize the conversion of any residual carbon magnetizations into antiphase carbon magnetization for spin systems with J^1_{CH} values different from the nominal value used to set the value of τ, and thereby achieve '*J*-compensation.'

In the present application of CAPT, we set τ' to a very short value (0.0001 s), thereby losing any '*J*-compensation,' and chose the phases of the pulses in order to acquire signals selectively from only some of the CH_n spin systems present in the sample while rejecting those from other spin systems. The coherence transfer diagrams given in Fig. 1 show that the pathway followed by the magnetization of quaternary carbons are also followed by some of the methylene carbon magnetization, and the pathways followed by the magnetization of methine carbons are also followed by some of the methyl carbon magnetization. It is, therefore, not surprising that one can obtain a subspectrum containing CH and CH_3 carbon resonances, or a subspectrum containing only resonances from C and CH_2 carbons, with no decrease in sensitivity. By cycling the phases for the proton pulses and the receiver in an appropriate manner, one can obtain subspectra which contain only quaternary carbon resonances (essentially blocking signals which are affected by the proton pulses), only methylene resonances, or only methyl resonances, but the last two have lower sensitivity than APT. One cannot generate a subspectrum which contains only methine carbon resonances by phase cycling. The phase cycles used to generate the various subspectra are given in Table 1. In addition to providing selective acquisition of appropriate subspectra, these phase cycles suppress artifacts due to pulse imperfections.

The 10–73 ppm regions of the natural abundance $^{13}C\{^1H\}$ CAPT spectra of 100 mg of cholesterol in 0.5 ml of $CDCl_3$ obtained with the phase cycles given in Table 1 are compared with the corresponding APT spectrum in Fig. 2. All spectra were obtained on a Bruker AM-300 spectrometer operating at a ^{13}C frequency of 75.469 MHz with a relaxation delay of 2 s between scans, 64 scans per spectrum (256 scans for the 'CH_2 only' spectrum and 264 for the 'CH_3 only' spectrum) and a Lorentzian line broadening of 1.2 Hz. Overall, the quality of the edited subspectra is very good, showing excellent suppression of unwanted resonances. The sensitivities of the 'C only,' 'C and CH_2' and 'CH and CH_3' subspectra are the same as that for the conventional multiplicity edited APT-type spectrum [Fig. 2(A)] since no pathways are blocked for the selected spin systems in the phase cycles used to generate these subspectra. However, the 'CH_2 only' and 'CH_3 only' subspectra have lower signal-to-noise (S/N) ratios because some of the CH_2 or CH_3 magnetization follows pathways which are blocked by the phase cycles.

Table 1. CAPT phase cycles for subspectra[a]

Spectrum/ phase	Edited[b]	CH and CH_3	C and CH_2	C only	CH_2 only	CH_3 only
ϕ_1	0	1 0 3 2	0	0	0	0
ϕ_2	0 2 0 2 3 1 3 1	1 0 1 0	0 0 1 1 2 2 3 3	0 2 0 2 3 1 3 1	0	0
ϕ_3	0 0 3 3	1 0 1 0	0 2 1 3	0 0 3 3	0	0
ϕ_4	1 3 3 1	0	2 0 1 3	2 0 0 2 0 2 2 0	0 1 2 3	(6) 0 1 2 3 4 5
ϕ_5	0	0 0 2 2	0	0	0	0
ϕ_6	1 3 3 1	2 0 0 2	0 0 1 1 2 2 3 3	1 3 3 1	0	0
ϕ_R	0 0 2 2 2 2 0 0	1 2 1 2	0	0 0 2 2 2 2 0 0	0 2	0 2

[a] Integers ϕ_i represent phase shift multiples of 90°, except for ϕ_4 in 'CH_3 only' which represents a phase shift multiple of 60°.

[b] Conventional APT-like multiplicity edited spectrum.

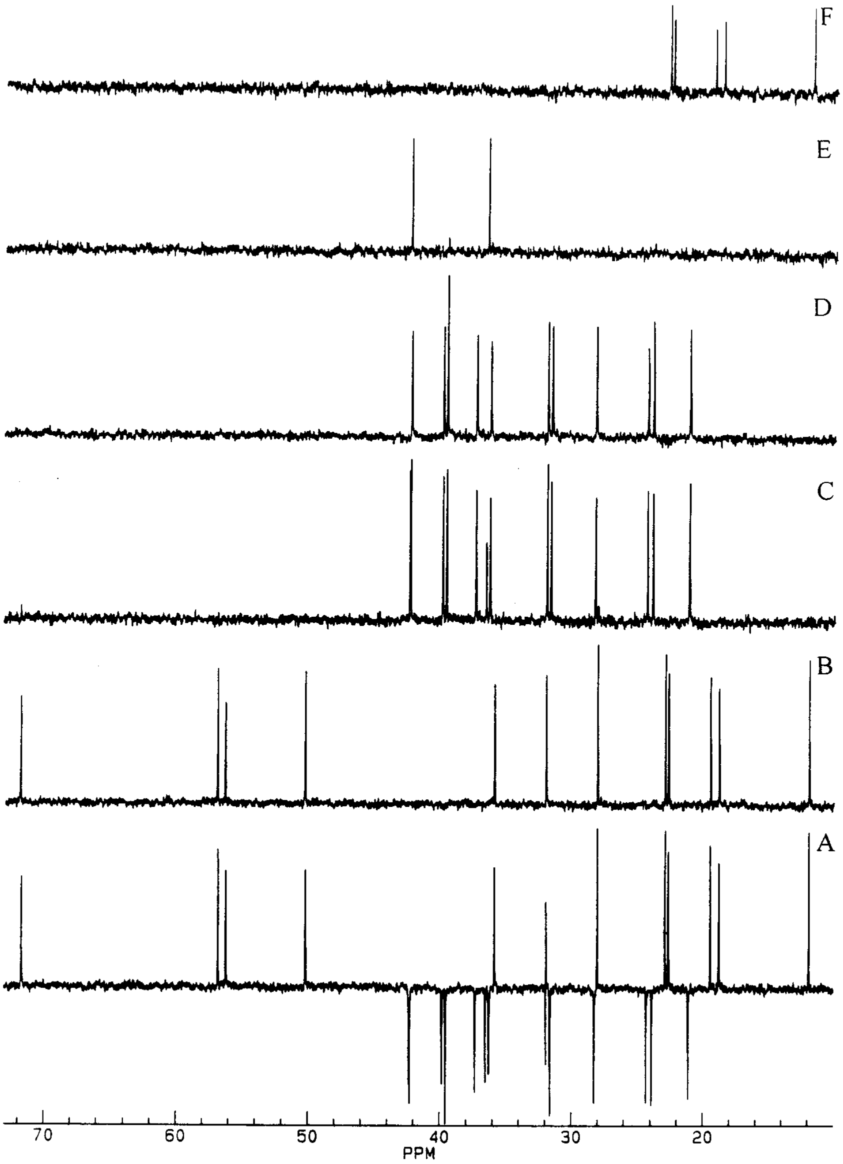

Figure 2. Comparison of the conventional APT multiplicity edited spectrum of cholesterol with CH and CH_3 carbon resonances up and the C and CH_2 carbon resonances down (A) with the CAPT subspectra obtained with phase cycles given in Table 1: 'CH and CH_3' (B); 'C and CH_2' (C); 'CH_2 only' (D); 'C only' (E), 'CH_3 only' (F).

For comparison, the APT 'CH and CH_3' and the 'C and CH_2' subspectra obtained by the addition and subtraction of the FID obtained with the proton decoupler gated off and the FID obtained with the decoupler left on during the 0.008 s spin-echo delay in the APT sequence[1] are shown in Fig. 3. There are a number of subtraction artifacts at a level of about 10% even though a line broadening of 3.5 Hz was applied to the FIDs. The incomplete cancellation of the resonances is probably due to very small differences in sample temperature in the presence and absence of decoupling. Addition and subtraction of CAPT spectra can be performed to obtain further subspectral editing. A 'CH_2 only' subspectrum generated by subtracting an appropriate multiple of the 'C only' subspectrum from the 'C and CH_2' subspectrum gave extremely good cancellation of quaternary carbon resonances, but this 'CH_2 only' subspectrum had a lower S/N ratio just as the 'CH_2 only' subspectrum generated directly [Fig. 2(D)] does. Similar results are shown in Fig. 4, where a 'CH only' subspectrum obtained by subtraction of an appropriate multiple of the 'CH_3 only' subspectrum from the 'CH and CH_3' subspectrum is compared with the 'CH and CH_3' subspectrum. Here the elimination of methyl resonances is effective, but small features remain at the positions of some of the methyl carbon resonances. This lower sensitivity suggests that a 'CH only' acquired with DEPT[4] using a 90° conversion pulse would be the preferred method.

Inverse 1D and 2D proton editing pulse sequences using conventional phase cycling procedures have been developed in which selection is achieved by variation of the delays in INEPT-type transfers,[9] variation of the flip angle of the final proton pulse in DEPT,[10] or multiple quantum filtering.[11] A number of gradient-based proton-detected editing sequences which use multiple

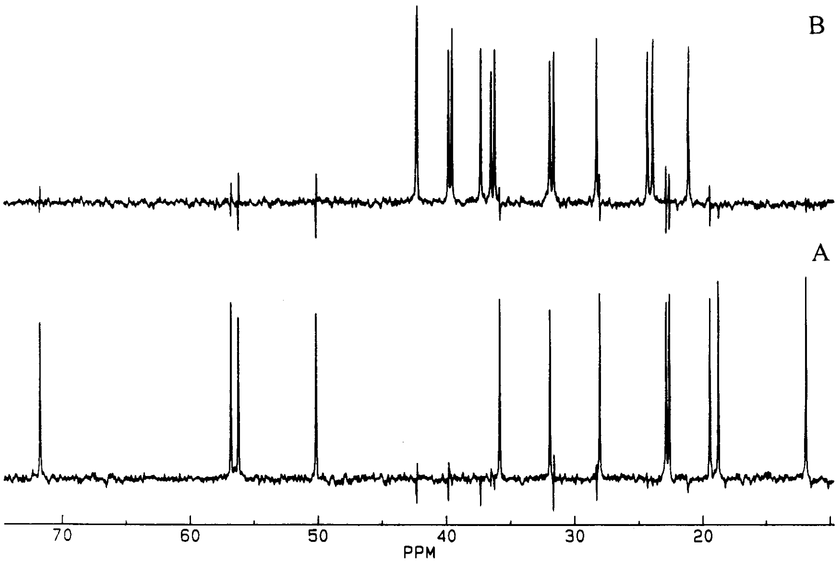

Figure 3. 'CH and CH_3' (A) and 'C and CH_2' (B) subspectra of cholesterol obtained by addition and subtraction of FIDs from APT experiments with the decoupler left on or gated off during the spin-echo delay (see text for details).

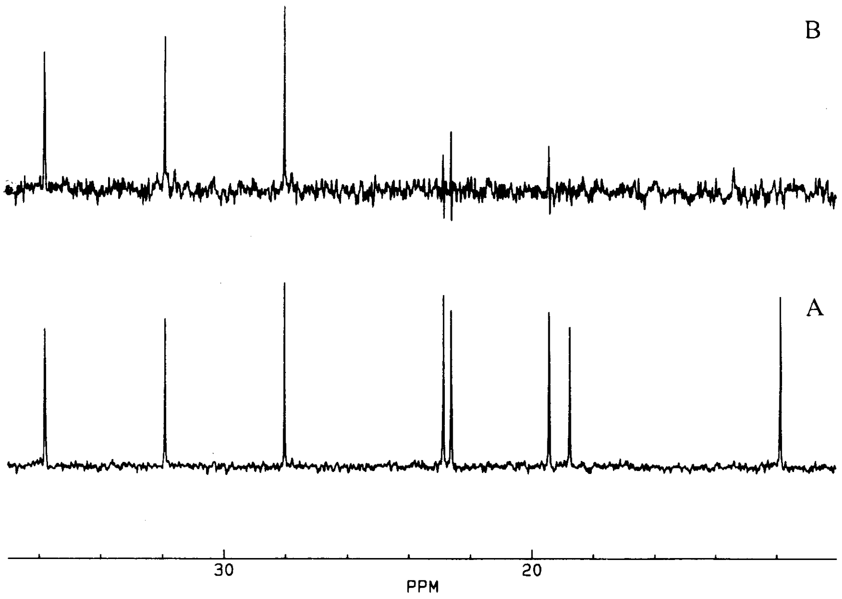

Figure 4. CAPT 'CH and CH_3' subspectrum (A) of cholesterol and a 'CH only' subspectrum (B) generated by subtracting an appropriate multiple of the 'CH_3 only' subspectrum.

quantum filtering have also been described.[12] In this paper we have shown that it is possible to generate exceptionally clean subspectra using multiple quantum filters with carbon detection without the need for the addition or subtraction of data. This approach should be of interest to users of older spectrometers which lack field gradients and inverse detection capabilities.

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