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Sensitivity-Enhanced Quantitative ^{13}C NMR Spectroscopy via Cancellation of $^{1}J_{CH}$ Dependence in DEPT Polarization Transfers

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Since the pioneering work of Schoolery[1] over 30 years ago, quantitative NMR spectroscopy has taken a prominent role in chemical analysis. When acquired under conditions allowing complete signal relaxation, ^{1}H spectra provide quantitative information for small molecules or simple mixtures of low-molecular weight compounds. However, as ^{1}H line widths increase with molecular weight, spectral signals often overlap, and signals of major components can obscure those of analytes at low concentrations. In these cases, deconvolution or single-value decomposition methods are required for quantitative analysis. A much simpler and typically preferred strategy is to use ^{13}C spectroscopy, as the broader range for ^{13}C chemical shifts affords much higher spectral resolution. Unfortunately, the low sensitivity of the ^{13}C nuclei, and very long relaxation delays necessary when at extreme narrowing conditions, typically make adequate signal-to-noise ratios very time-consuming to obtain.

Outside of simple means such as optimizing experimental temperature, ^{13}C sensitivity can be significantly enhanced by exploiting two different nuclear magnetic phenomena. The $^{13}C\{^{1}H\}$ nuclear Overhauser effect (NOE), a manifestation of $^{13}C-^{1}H$ dipolar cross-relaxation, can enhance ^{13}C sensitivity by as much as 299%.[2] Because cross-relaxation is intimately related to molecular dynamics, NOE enhancements can be vary dramatically from one molecule to the next. Moreover, enhancements are difficult to predict and cannot be controlled adequately for use in quantitative spectroscopy. Polarization transfer on the other hand, requiring scalar coupling between ^{1}H and ^{13}C nuclei ($^{1}J_{CH}$ coupling) in this case, can increase ^{13}C sensitivity by as much as $\gamma_{^{1}H}/\gamma_{^{13}C}$ or 398%. For pulse sequences incorporating polarization transfer steps such as INEPT, DEPT, and HSQC, delay periods are used to transfer magnetization between ^{1}H and ^{13}C nuclei. Invariably, just one delay period (Δ) optimized for a single $^{1}J_{CH}$-coupling is used; this is typically 145 Hz. Under these conditions, ^{13}C signal intensity is strongly dependent on $^{1}J_{CH}$ (see Figure 1), destroying any utility for polarization transfer in quantitative analysis. Reported herein is a scheme devised to cancel the signal intensity dependence on $^{1}J_{CH}$ in polarization transfers between ^{1}H and ^{13}C nuclei. The scheme has been incorporated into the DEPT pulse sequence to provide sensitivity-enhanced, quantitative DEPT (Q-DEPT) experiments.

The signal intensity J dependence of polarization transfers between ^{1}H and ^{13}C nuclei, described by eq 1,[3]

$$I \propto \sin^2(\pi\Delta^{1}J_{CH}) \tag{1}$$

and illustrated in Figure 1 optimized for $^{1}J_{CH} = 145$ Hz, can result in signal intensity differences of up to ca. 240%. The devised scheme is based on the summation of time domain data acquired with suitably selected Δ values so that all J dependence is virtually eliminated in the final, signal-averaged, free induction decay. The Δ values were determined iteratively by minimizing the difference between maximum and minimum I over the natural $^{1}J_{CH}$ range (115−220 Hz), an approach used to design quantitative HSQC spectroscopy.[3] Iterations were constrained to all $\Delta < 6$ ms to reduce spin−spin relaxation losses and evolution of homonuclear couplings during polarization transfers. Four Δ values; 2.67, 3.11, 3.12, and 5.96 ms, were found to average the span of signal intensity from $^{1}H-^{13}C$ polarization transfers to only 1.5% over the natural $^{1}J_{CH}$ range (Figure 1). Attempts using two values were not successful, and eight gave no improvement.

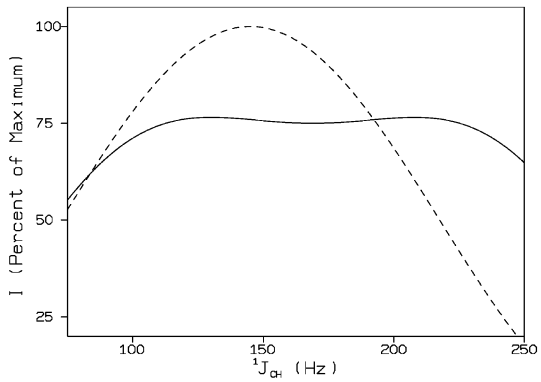

Figure 1. Simulated J dependence of DEPT (broken line) and Q-DEPT (solid line) spectroscopy.

Figure 2 shows the data averaging scheme incorporated into the DEPT pulse sequence.[5] The angle θ of the read pulse generates both the intensity and positive or negative phase of all signals in the resulting spectrum. This dependency on θ is unique for ^{13}C nuclei directly bonded to one, two, or three ^{1}H nuclei, and is described by eqs 2, 3, and 4,[6] respectively:

$$I_{CH} = (\gamma_{^{1}H}/\gamma_{^{13}C}) \sin\theta \tag{2}$$

$$I_{CH2} = (\gamma_{^{1}H}/\gamma_{^{13}C}) \sin 2\theta \tag{3}$$

$$I_{CH3} = [3\gamma_{^{1}H}/4\gamma_{^{13}C}] (\sin\theta + \sin 3\theta) \tag{4}$$

For quaternary carbon atoms, the absence of a directly bonded ^{1}H nucleus prevents polarization transfer and a corresponding signal in DEPT spectra. Any two of the three equations can be solved simultaneously for θ to find a read pulse giving the same intensity and phase for their respective type of carbon atom. The third type will have an intensity and phase described by the remaining equation when solved for the derived θ. For example, eqs 2 and 3 can be solved simultaneously to find that a 60° read pulse gives the same signal intensity for methine and methylene ^{13}C nuclei, then eq 4 can then be solved for $\theta = 60°$ to find the relative intensity of methyl ^{13}C signals. Reported in Table 1 (experiments 1−3) are the results of solving the equations for all pairwise combinations of two. The table also lists the Q-DEPT theoretical sensitivity

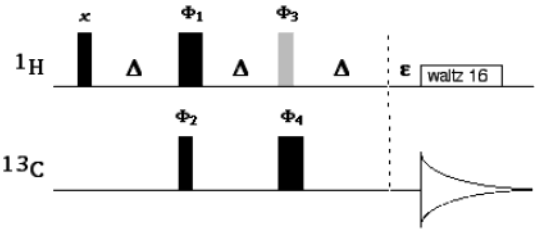

Figure 2. Q-DEPT pulse sequence. Thin and thick black bars are 90 and 180° pulses, respectively, and the read pulse is the gray bar. Δ delays are cycled 12(2.67 ms), 12(3.11 ms), 12(5.96 ms), 12(3.12 ms) so that each acquisition is recorded with three delays of the same value. The delay ϵ compensates for chemical shift evolution during pulses. The phase of the first pulse is held at x, while all other pulses and the receiver, are phase-cycled as follows: $\Phi_1 = x, -x, y, -y$; $\Phi_2 = 8(x), 8(-y), 8(-x), 8(-y)$; $\Phi_3 = 4(y), 4(-y)$; $\Phi_4 = 4(x, -x), 4(y, -y)$; $\Phi_{rcv} = 2(y), 4(-y), 2(y), 2(-x), 4(x), 2(-x), 2(-y), 4(y), 2(-y), 2(x), 4(-x), 2(x)$. For the uniform sensitivity enhancement experiment (Table 1, experiment 4), θ are cycled 4(50.0°), 4(35.6°), 4(49.1°), 4(84.9°). ^{1}H decoupling is by the waltz 16 sequence.[4]

Table 1. Read Pulse Angles and Sensitivity Enhancements for Q-DEPT Spectroscopy

		sensitivity enhancement (%)[c]		
experiment	θ (deg)[a,b]	CH	CH_2	CH_3
1	45.0	213	301	301
2	54.8	246	284	246
3	60.0	261	261	196
4	$(50.0 + 35.6 + 49.1 + 84.9)_n$	234	234	234

[a] Read pulse angle in degrees. [b] All θ are ≤90° for suppression of off-resonance effects. [c] Theoretical sensitivity enhancements relative to conventional quantitative $^{13}C\{^1H\}$ spectroscopy, for signals of carbon atoms with one (CH), two (CH_2), or three (CH_3) directly bonded protons.

enhancements for all protonated carbon atoms relative to conventional quantitative $^{13}C\{^1H\}$ spectroscopy.[7] A set of four additional θ values appearing in the table (experiment 4) is a series of read pulse angles that, when cycled together with the data averaging scheme (see Figure 2 caption), give spectra with uniform sensitivity enhancements for all detectable ^{13}C signals. These pulse angles were also found iteratively; eqs 2, 3, and 4 were solved simultaneously for four adjustable θ values until they converged on the same, pre-assigned value of signal intensity. This value was incremented, and iteration continued. The entire process was repeated until convergence was no longer possible. Attempts using eight θ values gave only a marginal (ca. 1%) enhancement in sensitivity.

Table 1 reveals that Q-DEPT spectroscopy should be about 200−300% more sensitive than its conventional counterpart. While 25% lower than routine DEPT spectroscopy with Δ delays optimized for specific signals, substantial reductions in acquisition times can still be realized using the quantitative experiments. For any one sample, Q-DEPT spectra will have similar signal-to-noise ratios as conventional quantitative ^{13}C spectra acquired 4−9 times longer[8] under the same conditions.

Finally, to confirm the ability of Q-DEPT spectroscopy for providing quantitative results, three spectra were collected using each of the four experiments for 90% ethylbenzene ($C_6H_5CH_2CH_3$) in acetone-d_6. Three spectra were also collected with conventional quantitative $^{13}C\{^1H\}$ experiments for comparison. Experiments were conducted at 125.77 MHz under conditions allowing complete signal relaxation and no cross-relaxation. The data sets, consisting of 65 536 complex points, 221 ppm spectral windows and 512 acquisitions, were multiplied by a 5 Hz line broadening factor before Fourier transformation into spectra. Table 2 summarizes the results

Table 2. Quantitative Results for Ethylbenzene Using Q-DEPT and Conventional $^{13}C\{^1H\}$ Spectroscopy[a]

	Q-DEPT experiment[b]				
signal	1	2	3	4	$^{13}C\{^1H\}$[c]
CH_3	0.96 ± 0.01	1.05 ± 0.07	0.76 ± 0.01	0.95 ± 0.03	0.96 ± 0.05
p-CH	0.72 ± 0.01	0.91 ± 0.02	1.00 ± 0.07	1.09 ± 0.01	0.96 ± 0.04
m-CH	0.72 ± 0.02	0.91 ± 0.01	1.02 ± 0.04	1.09 ± 0.08	0.98 ± 0.09
o-CH	0.73 ± 0.03	0.92 ± 0.01	1.01 ± 0.04	1.08 ± 0.09	1.01 ± 0.05

[a] Table entries are from the integral values of ethylbenzene ^{13}C signals for the methyl group (CH_3) and the carbon atoms para (*p*-CH), meta (*m*-CH), and ortho (*o*-CH) to the ethyl group. Integrals are normalized to the methylene signal, assigned a value of 1.00 in all but experiment 2 spectra. Integrals of experiment 2 methylene signals were assigned a value of 284/246 or 1.15, derived from the sensitivity enhancements in Table 1. For simplicity, integral values were calculated to represent one carbon atom; i.e., *m*-CH signal integrals were divided by 2, and those of CH_3, by 1. Entries are reported ±95% confidence intervals. [b] Experiments in Table 1. [c] quantitative ^{13}C observation with inverse-gated ^1H decoupling.

of integrating the ethylbenzene signals. As anticipated, all signals, except those for aromatic ^{13}C from experiment 1 and the methyl signals from experiment 3, have integral values very close to 1.00 when normalized to represent a single carbon atom. When these integrals are corrected for the intensity differences expected from their theoretical enhancements (Table 1), they have normalized values very close to 1.00 as well (not shown). In addition, experiment 4 integrals are all very similar to their corresponding integrals from the conventional experiments, a direct consequence of the θ cycling scheme equalizing the sensitivity enhancements of all protonated ^{13}C atoms. After adjusting for the expected differences in sensitivity then, little difference is evident between the spectra from all Q-DEPT and conventional quantitative spectroscopy.

In conclusion, polarization transfer delays in the DEPT pulse sequence have been modulated to create sensitivity-enhanced experiments for collecting quantitative $^{13}C\{^1H\}$ spectra. Four experiments, each with unique read pulse angles, give quantitative spectra with 200−300% more sensitivity than corresponding conventional methods.[7] The experiments can be used to acquire spectra with improved quantitative information or to substantially reduce the long acquisition times indicative of quantitative ^{13}C experiments. While the ability of the experiments to provide quantitative spectra was confirmed with ethylbenzene, they can easily be adapted to analyze complex mixtures.

Supporting Information Available: $^{13}C\{^1H\}$ spectra of 45% ethylbenzene in acetone-d_6 acquired using Q-DEPT and conventional quantitative spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Signal-to-noise ratio is directly proportional to the square root of the number of data acquisitions collected.

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