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Sensitivity Enhancement of ^{13}C T_1 Measurements via Polarization Transfer

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Indirect detection pulse schemes have proven extremely valuable for the observation of low sensitivity nuclei (X nuclei) (*1–6*). Different approaches have been provided by Müller (*2*) and by Bodenhausen and Ruben (*1*). In the first case two-spin (^1H–X) multiple-quantum polarization is established and detected through the proton nucleus so that the sensitivity of the experiment is independent of the gyromagnetic ratio of X. In the latter case transfer of ^1H polarization to X is accomplished via the INEPT pulse sequence (*7*). After a fixed evolution time X coherence is indirectly detected through the more sensitive ^1H nucleus by transferring coherence back to ^1H by a reverse INEPT sequence (*8*).

Most applications of the above sequences have focused on chemical-shift measurements or correlation of X chemical shifts with ^1H chemical shifts for assignment purposes. In this communication, however, we concentrate on indirect detection as a means of making ^{13}C NMR relaxation studies more practical. In principle, ^{13}C NMR relaxation studies can provide a detailed description of molecular dynamics (*9*). Such a description is possible since ^{13}C relaxation is dominated by dipolar interactions between the ^{13}C nucleus and directly bonded protons (*10*) and since the ^1H–^{13}C bond distance is known accurately (*10, 11*). However, the inherent insensitivity of ^{13}C has meant that ^{13}C relaxation studies have required large sample volumes, substantial concentrations, and large acquisition times. We have developed several pulse sequences which improve the sensitivity of ^{13}C relaxation experiments. These sequences are modifications of the Bodenhausen and Ruben sequence (*1*) in that ^{13}C magnetization is indirectly detected through bonded ^1H nuclei via polarization transfer. We provide, first, a theoretical description of the pulse sequences followed by an application to the measurement of a spin–lattice relaxation time, T_1, for $^{13}C_\beta$ of 99% ^{13}C enriched alanine.

Figure 1 provides two examples of pulse sequences that we have used for measuring ^{13}C T_1's with improved sensitivity. Both sequences incorporate DEPT pulse schemes (*12, 13*), which allows transfer of polarization between ^{13}C and ^1H. While in principle other polarization transfer schemes such as INEPT (*7*) can be used in place of DEPT, we prefer the latter sequence since it involves fewer pulses (albeit at the expense of

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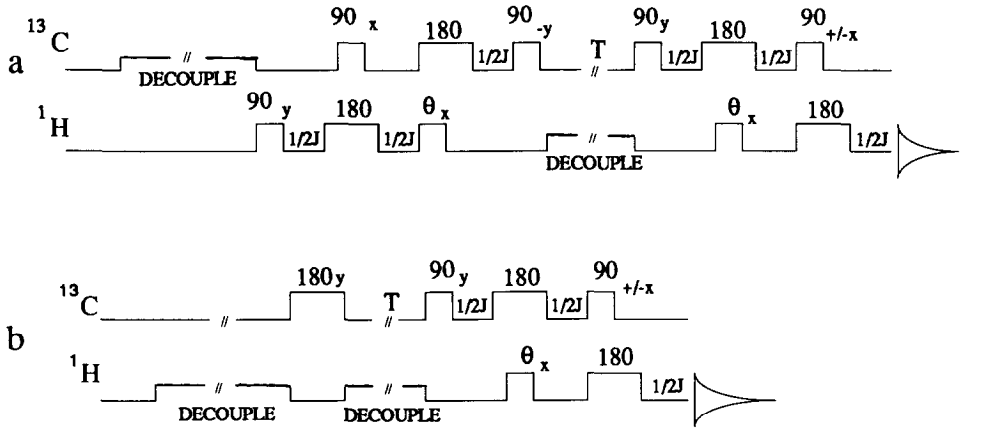

FIG. 1. Pulse sequences used to measure ^{13}C T_1's. Sequence (a) transfers ^1H polarization to ^{13}C and then back to ^1H for observation so that the sensitivity of the signal should be independent of the carbon gyromagnetic ratio. Sequence (b) transfers polarization originating on ^{13}C to ^1H for detection and hence is less sensitive. The sequences are not drawn to scale.

being slightly longer). In addition the DEPT sequence has been shown to produce less artifacts than the INEPT sequence when a range of ^{13}C–^1H couplings are present (*13*).

The pulse sequences can be readily understood by using the product operator formalism of Sørensen *et al.* (*14*). In particular we focus on the sequence shown in part a of Fig. 1. For simplicity we shall consider an AX spin system and neglect transverse relaxation effects. The density operator before application of ^{13}C decoupling can be written as

$$\rho \propto \omega_H I_z + \omega_C S_z, \qquad [1]$$

where I_z and S_z are z spin operators associated with the proton and carbon spins, respectively, and ω_i is the Larmor frequency of nucleus i. ^{13}C saturation equalizes the populations of the S energy levels so that $S_z = 0$ and accordingly we write that

$$\rho \propto \omega_H I_z, \qquad [2]$$

where we have neglected the small NOE effect. To simplify the algebra without losing anything essential in what follows we assume that both ^{13}C and ^1H spins are on resonance. Application of a 90°_y ^1H pulse gives

$$\rho \propto \omega_H I_x. \qquad [3]$$

Evolution for $1/2J$, where J is the ^{13}C–^1H geminal coupling constant, followed by simultaneous application of 90°_x (^{13}C) and 180°_y (^1H) pulses gives rise to two-spin polarization according to

$$\rho \propto -2\omega_H I_y S_y. \qquad [4]$$

Evolution for a second $1/2J$ period, subsequent application of 180°_x (^{13}C) and 90°_x (^1H) pulses ($\theta = 90°$ for an AX spin system (*12*)), followed by an additional evolution period of $1/2J$ produces

$$\rho \propto -\omega_H S_x. \qquad [5]$$

Application of a $90°_{-y}$ ^{13}C pulse gives

$$\rho \propto -\omega_H S_z. \quad [6]$$

Equation [6] states that polarization has been transferred from ^1H to ^{13}C and at this stage a factor of 4 increase in sensitivity is obtained over the conventional ^{13}C T_1 experiment in the absence of initial saturation of proton spins.

Relaxation proceeds for a time T and can be described using the Solomon equations (*15*) as

$$\frac{dM_z^I}{dt} = -R_{II}(M_z^I - M_{z,0}^I) - \sigma_{IS}(M_z^S - M_{z,0}^S)$$

$$\frac{dM_z^S}{dt} = -R_{SS}(M_z^S - M_{z,0}^S) - \sigma_{SI}(M_z^I - M_{z,0}^I), \quad [7]$$

where M_z^I and M_z^S are the z components of magnetization associated with spins I and S, respectively, $M_{z,0}^I$ and $M_{z,0}^S$ are the equilibrium components of I and S magnetization, $R_{II} = W_0 + 2W_{1I} + W_2$, $R_{SS} = W_0 + 2W_{1S} + W_2$, $\sigma_{IS} = \sigma_{SI} = W_2 - W_0$, and W_2, W_0, W_{1I}, and W_{1S} denote double-quantum, zero-quantum, and one-quantum I and S spin-transition probabilities, respectively (*15*). For the case of ^1H decoupling, the equation describing the evolution of M_z^S reduces to

$$\frac{dM_z^S}{dt} = -R_{SS}(M_z^S - (1+\eta)M_{z,0}^S), \quad [8]$$

where $\eta = (\sigma_{SI}/\rho_S)(\gamma_I/\gamma_S)$ and we have used the relation $M_{z,0}^I = (\gamma_I/\gamma_S)M_{z,0}^S$. Equation [8] shows that the evolution of M_z^S under these conditions is monoexponential and proceeds to an equilibrium value $(1 + \eta)M_{z,0}^S$ according to

$$M_z^S(t) = (1+\eta)M_{z,0}^S + (M_z^S(0) - (1+\eta)M_{z,0}^S)\exp(-R_{SS}t), \quad [9]$$

where $M_z^S(0)$ is the intensity of the ^{13}C signal immediately following the $90°_{-y}$ ^{13}C pulse and $R_{SS} = 1/T_{1S}$, where T_{1S} is the T_1 of spin S. After a relaxation time $t = T$ we can write

$$\rho \propto -\omega_H S_z f(T), \quad [10]$$

where $f(t)$ describes the relaxation of $M_z^S(t)$ according to Eq. [9] (i.e., $f(t) \propto (1 + \eta) - (\gamma_I/\gamma_S + (1 + \eta))\exp(-R_{SS}t)$ where we have replaced $M_z^S(0)$ by the appropriate initial condition, $M_z^S(0) = -\gamma_I/\gamma_S M_{z,0}^S$).

The final set of pulses transports magnetization from ^{13}C back to ^1H for detection. Application of a $90°_y$ ^{13}C pulse, evolution for $1/2J$, subsequent application of $180°_y$ (^{13}C) and $\theta = 90°_x$ (^1H) pulses followed by an additional evolution period of $1/2J$ yields

$$\rho \propto 2\omega_H I_y S_y f(T). \quad [11]$$

Finally, application of simultaneous $90°_x$ (^{13}C), $180°_y$ (^1H) pulses followed by evolution for $1/2J$ gives

$$\rho \propto -\omega_H I_X f(T). \quad [12]$$

Since ^1H magnetization is detected an additional increase in sensitivity of $(\gamma_I/\gamma_S)^{3/2} = 8$ is obtained over ^{13}C detection experiments.

Inspection of Eqs. [9] and [12] shows that a plot of $\ln[(A(t) - A(\infty))/(A(0) - A(\infty))]$ vs t, where $A(t)$ is the integrated ^{1}H signal at time t, will yield a straight line with a slope of $1/T_{1S}$.

Figure 1b shows an alternate scheme for ^{13}C T_1 measurements. This sequence is less sensitive than the sequence described in Fig. 1a since in this case polarization originates on ^{13}C and is transferred to ^{1}H for detection. However, the NOE effect which occurs due to the saturation of the ^{1}H spins at the outset of this experiment can increase the sensitivity.

A product operator calculation similar to the one shown above indicates that in the limit of perfect pulses, with neglect of losses due to transverse magnetization, and for $t < 1/R_{SS}$, the ratio of signal intensities expected from the sequences of Figs. 1b and 1a is

$$\frac{(1+\eta)(\gamma_S/\gamma_I)}{N \sin\theta \cos^{N-1}\theta}, \qquad [13]$$

where N is the number of equivalent proton spins bound to the carbon nucleus and θ is defined in Fig. 1. Figure 2 shows a plot of this ratio as a function of $\omega\tau_c$ for an AX spin system executing isotropic motion with a correlation time of τ_c in the limit of $t < 1/R_{SS}$. The ratio of signal intensities that can be expected from a ^{13}C inversion-recovery sequence with proton presaturation and the sequence of Fig. 1a as a function of $\omega\tau_c$ can also be obtained from Fig. 2 by dividing the ordinate axis by 8.

Inspection of Fig. 2 shows that the sequence of Fig. 1a is particularly appealing for application to large molecules where the ^{13}C–^{1}H NOE effect is small (*16*). However, the large number of pulses involved and the increase in time required to execute the sequence may reduce some of its sensitivity advantages. In particular, for macromolecules where transverse relaxation effects cannot be neglected, magnetization will be lost.

One potential disadvantage associated with these indirect detection methods is interference from protons not directly bound to ^{13}C. We have achieved suppression of unwanted ^{1}H magnetization by alternating the phase of the final 90° carbon pulse

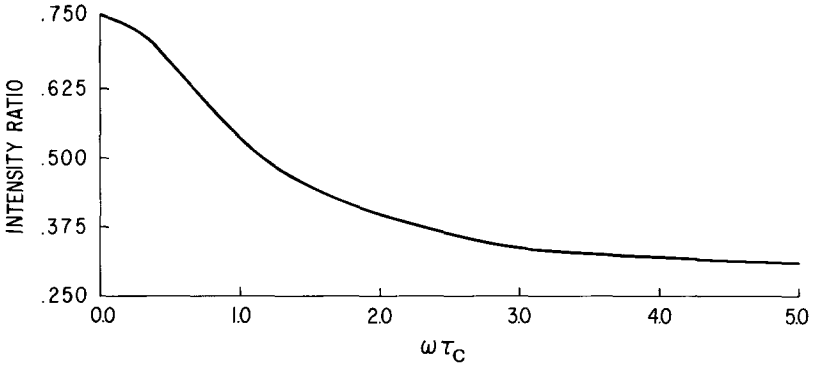

FIG. 2. Plot of the signal intensity expected for the sequence of Fig. 1b relative to the intensity expected for the sequence of Fig. 1a as a function of $\omega\tau_c$ for an AX spin system in the limit of perfect pulses and neglecting transverse relaxation.

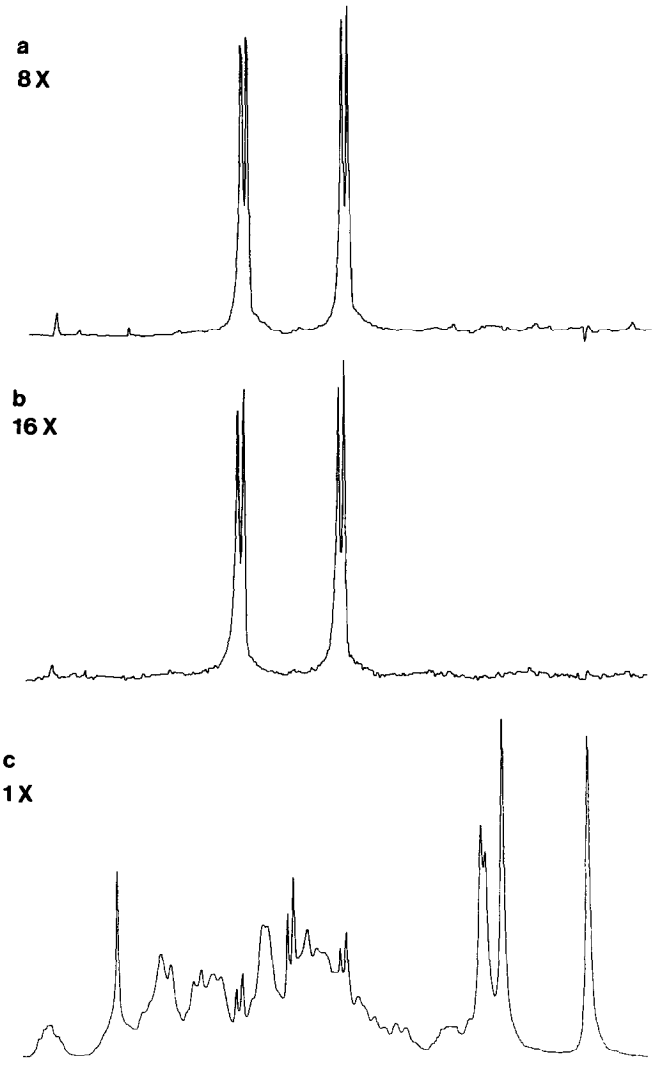

FIG. 3. Suppression levels expected from the sequences of Fig. 1. (c) shows a spectra of 25 m*M* deoxycholate, 10 m*M* alanine (natural abundance), 10 m*M* acetate (natural abundance), and 10 m*M* 99% $^{13}C_{\beta}$ CH_3 alanine recorded after 8 scans on a home-built 490 MHz spectrometer operating in the Fourier transform mode. The separation between ^{13}C satellites is 131 Hz. Each satellite is split into a doublet due to an αCH–βCH_3 ^1H–^1H coupling of 7 Hz. (a) Spectra obtained with the sequence of Fig. 1a after 16 scans (T = 200 ms). All other acquisition parameters are the same as in (c). (b) Spectra obtained with the sequence of Fig. 1b with 15 s ^1H presaturation. All parameters are the same as in (a) and (c).

and subtracting the resulting signal (*1*). In the case of the sequence indicated in Fig. 1b, suppression is enhanced by presaturation of the proton resonances (*8*). Figure 3 illustrates the suppression levels obtained by both sequences with a sample consisting of 25 m*M* deoxycholate, 10 m*M* alanine (99% ^{13}C βCH_3), 10 m*M* alanine (natural

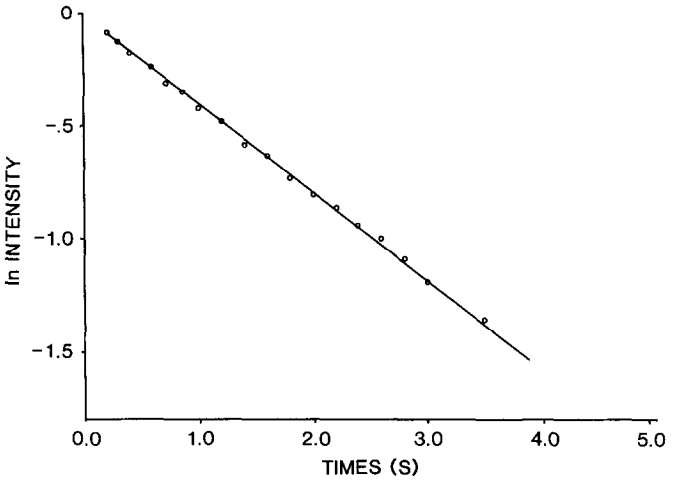

FIG. 4. Magnetization profile as a function of time (T) obtained with the sequence of Fig. 1a on a sample of 10 mM 99% $^{13}C_\beta$ alanine. Intensity = $[A(t) - A(\infty)]/[A(0) - A(\infty)]$, where $A(t)$ is the integrated proton signal at time t.

abundance ^{13}C), and 10 mM acetate (natural abundance ^{13}C). The suppression and sensitivity displayed seem quite encouraging for application to macromolecules.

The sequences described in Fig. 1 were used to measure the $^{13}C_\beta$ T_1 of alanine and show good agreement with T_1 results obtained using the conventional ^{13}C inversion recovery technique followed by ^{13}C observation. Figure 4 shows a magnetization versus time profile obtained with the sequence of Fig. 1a. Table 1 lists the T_1's obtained from the three different methods employed.

Recently, an alternate method for obtaining ^{13}C T_1's via indirect detection through protons has been suggested (*17*). This technique requires both ^{13}C enriched and natural abundance samples. The increase in relaxation rates that the protons bound to a ^{13}C nucleus show in the enriched sample relative to the rates in the natural abundance sample can be used to estimate the carbon T_1. However, this technique suffers from the fact that both ^{13}C and ^{12}C samples are required. In addition, since the ^{13}C relaxation rates obtained are the result of differences in ^1H T_1's of enriched and natural abundance

TABLE 1

Comparison of T_1 Values

Method	T_1 (s)[a]
^{13}C inversion recovery with ^1H presaturation	2.3 ± 5%
Sequence of Fig. 1a	2.6 ± 5%
Sequence of Fig. 1b	2.3 ± 5%

[a] T_1 values calculated by a least-squares fit of the data.

samples the errors associated with this method tend to be significant. For these reasons we prefer measurement of ^{13}C T_1's via the polarization transfer method.

In summary, we have presented several pulse sequences which improve the sensitivity of ^{13}C T_1 relaxation measurements. It is anticipated that these sequences will be useful for the measurement of ^{13}C T_1's of specifically labeled residues in macromolecules, where the NOE effect is negligible (*16*) and where conventional techniques require concentration levels and acquisition times that are often impractical.

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