Fourier Transform Study of NMR Spin–Lattice Relaxation by "Progressive Saturation"

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When a nuclear spin system is subjected to a repetitive sequence of strong radiofrequency pulses, a steady state is established where there is a dynamic balance between the effect of the pulses and spin relaxation. Under certain readily satisfied pulse conditions, the deviation of the intensity of the free induction signal from its thermal equilibrium value is an exponential function of the pulse interval with time constant equal to the spin–lattice relaxation time. The determination is unaffected by spin–spin relaxation provided that the interval between pulses is long enough to permit all transverse components of magnetization to be eliminated, and provided precautions are taken to inhibit spin–echo formation. Through Fourier transformation of the transient response, high resolution spectra with many component resonances may be studied, and the spin–lattice relaxation times of the individual lines determined. The technique lends itself particularly well to repeated accumulation of the transient signal for the purpose of improving sensitivity. It has been applied to the problem of determining the spin–lattice relaxation rates of the eight different carbon-13 resonances in 3,5-dimethylcyclohex-2-ene-1-one. The results span a range from 2.6 to 39 sec, and are in good agreement with those obtained by applying $180°-t-90°$ sequences to the same sample.

I. INTRODUCTION

In conventional nuclear magnetic resonance spectroscopy one of the simplest ways to study relaxation effects is the "progressive saturation" technique, where the signal peak height is monitored as a function of the applied radiofrequency field H_1. Since the experiment excites a finite transverse component of magnetization, spin–spin as well as spin–lattice relaxation is involved, and the measurement is commonly used to derive the spin–lattice relaxation time in situations where the spin–spin relaxation time is known from other experiments, or is known to be equal to the spin–lattice relaxation time. The normal procedure is to determine the radiofrequency level which yields the maximum signal peak height; to derive the relaxation information this radiofrequency level must be calibrated in frequency units. Inhomogeneous broadening of the line, through, for example, nonuniformity of the applied static field, modifies the saturation curve[1] and complicates the determination.

An analog of the progressive saturation experiment can also be carried out in a pulse-excited mode. A repetitive train of strong radiofrequency pulses is applied to the nuclear spin system, and a steady state established where there is a dynamic balance between excitation and relaxation between pulses. It can be shown that if the pulse parameters are adjusted to the condition where the spin magnetization is nutated into the transverse (XY) plane, and if all spin isochromats are destroyed (or scrambled in such a way as to prevent echo formation) before each pulse, the spin dynamics are determined by spin–lattice relaxation only, with no complications due to spin–spin relaxation.

In the steady state, the amplitude of the free precession signal is directly proportional to the longitudinal component of magnetization that has recovered in the interval t between pulses. Recovery is essentially complete if the pulse interval is made long in comparison with the spin–lattice relaxation time, and the free precession signal reaches its thermal equilibrium value, S_∞. When the signal intensity is studied as a function of the pulse interval, the difference $(S_\infty - S_t)$ follows an exponential with time constant equal to the spin–lattice relaxation time, provided certain operating conditions are satisfied. The experiment thus consists of a series of measurements of signal amplitude at different settings of the pulse repetition rate, with at least one measurement at a pulse separation that is large compared with all spin–lattice relaxation times, to give the thermal equilibrium value.

Fourier transformation of the transient free precession signal gives the conventional slow-passage spectrum[2,3] so that it is particularly interesting to apply this technique under high resolution conditions to molecules containing several nonequivalent nuclei, in order to obtain multiline spectra where the spin–lattice relaxation time of each individual line may be monitored selectively. The regime of a standard Fourier transform spectrometer requires remarkably little modification for this experiment. The pulse length must be adjusted to the condition where all magnetization components are essentially transverse after the pulse (for pulses that are very strong in comparison with the frequency width of the spectrum, this corresponds to the 90° pulse condition). Spin-echo formation must be inhibited such that net transverse components of magnetization are negligible at the end of the free precession period with no possibility of refocussing. A variable waiting period must be provided after the transient signal has been sampled and before the next pulse. Provision must be made for the establishment of steady-state conditions before any free precession signals are acquired, a precaution that is normally ignored in conventional Fourier transform work. None of these requirements makes critical demands on the capacity of a typical high resolution Fourier transform spectrometer.

The proposed technique naturally invites comparison with the classic "inversion recovery" method[4,5] of measuring T_1, where an initial 180° pulse inverts all nuclear spin populations, which then recover towards thermal equilibrium and are monitored after a time t by a 90° pulse which excites the transient free precession signal. (Through Fourier transformation both techniques can be applied to the measurement of individual lines of a high resolution spectrum.) One drawback of the inversion recovery method is the requirement that time be allowed for the spins to reach thermal equilibrium before the reapplication of the 180° pulse, thus each measurement of the transient free induction signal must be separated from the next by a waiting period that is long compared with the longest spin–lattice relaxation time under investigation, $T_1(\max)$. A rough estimate of $T_1(\max)$ must therefore be made before the experiment can be set up, and there are penalties if the estimate is too short (the longer relaxation times will be underestimated) or too long (sensitivity will be sacrificed). Although both techniques eventually require an estimate of $T_1(\max)$ in order to determine the thermal equilibrium spectrum, the progressive saturation experiment can proceed without this information, gathering data that will indicate a suitable value for $T_1(\max)$. When extensive time averaging is used with the progressive saturation technique, the time taken to establish the steady state, and the time used for transformation and recording the spectrum are negligible with respect to the actual accumulation of signals. This steady-state experiment thus accumulates transient data much faster than the inversion–recovery method. However the latter recovers a large part of this sensitivity advantage because it inverts rather than equalizes the spin populations, thus benefitting from a twofold increase in signal intensity. The net result is that both methods achieve a sensitivity of the same order, although the progressive saturation experiment is favored for qualitative experiments aimed at demonstrating the general trend of relaxation rates.

II. THEORY

The steady-state behavior of an isolated nuclear spin subjected to a repetitive sequence of radiofrequency (rf) pulses has been analyzed in detail by Ernst and Anderson.[3] This treatment is extended here to cover the effects of finite pulse strength, to consider the conditions necessary for eliminating transverse components of magnetization before each pulse, and to investigate the rate at which the steady-state response to the pulses is established. This involves a consideration of the damping of the free induction decay by static field inhomogeneity effects, and the possibility of refocussing by the rf pulses.

A. Effects of Finite Pulse Strength

In a practical Fourier transform spectrometer operating in the high resolution mode, the distribution of chemical shift values will often cover such a wide frequency range (ΔF Hz) that it is not practicable to satisfy the condition for an "ideal" rf pulse:

$$\gamma H_1/2\pi \gg \Delta F. \qquad (1)$$

Consider a typical magnetization component ΔH gauss from exact resonance. In a frame of reference rotating about the direction of the static field (the $+Z$ axis) at an angular frequency $\omega_0 = \gamma H_0$, this component will experience an effective field H_{eff} given by

$$H_{\mathrm{eff}} = (H_1{}^2 + \Delta H^2)^{1/2}. \qquad (2)$$

The direction of H_{eff} is tilted at an angle ϕ with respect to the X axis in the XZ plane of the rotating frame (see Fig. 1) where

$$\tan\phi = \Delta H/H_1. \qquad (3)$$

If τ is the rf pulse width in seconds, a magnetization vector at exact resonance will be nutated through an angle α about the X axis, where

$$\alpha = \gamma H_1 \tau. \qquad (4)$$

Off-resonance vectors will be nutated through larger angles ψ about the tilted axis of H_{eff},

$$\psi = \gamma H_{\mathrm{eff}}\tau = \alpha/\cos\phi. \qquad (5)$$

The present concern is with nominal flip angles α equal to $\pi/2$ radians, such that an "ideal" rf pulse would nutate all vectors from the $+Z$ direction into the $+Y$ direction. Figure 1 illustrates the result for pulses of
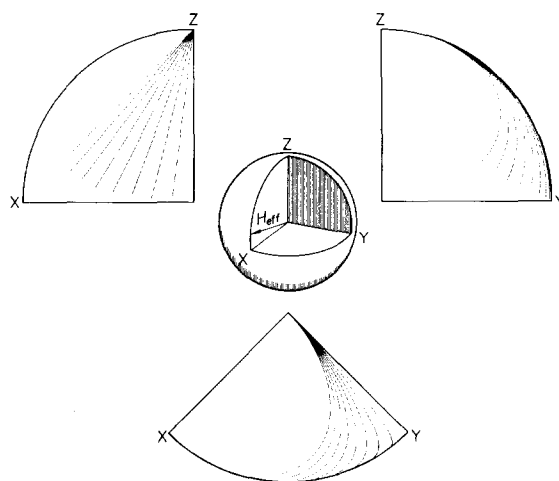

FIG. 1. The effect of a "90°" pulse of finite strength H_1 on magnetization components displaced from the exact resonance condition a distance ΔH gauss. The trajectories of a unit magnetization vector as it rotates about H_{eff} have been calculated for a series of values of $(\Delta H/H_1)$ ranging from zero to unity in eight equal steps. Loci of the tip of this vector are shown in projection on the XZ, YZ, and XY planes of the rotating frame of reference. Note that the transverse component of magnetization after the pulse is always very close to unity, and exhibits a dephasing with respect to the Y axis which is to a good approximation a linear function of ΔH. For sufficiently small values of the offset parameter $(\Delta H/H_1)$ the residual longitudinal (Z) component of magnetization after the pulse is negligibly small.

finite strength for a family of typical vectors representing a range of values of the parameter $(\Delta H/H_1)$ from zero to unity. Because the effect of tilting the axis of H_{eff} is to some extent compensated by the increase in flip angle ψ, the vectors end up very close to the XY plane with transverse components of magnetization very near unity, for small values of $(\Delta H/H_1)$. However the off-resonance vectors spread out appreciably in the XY plane, and if a free precession signal were to be excited in this way and then subjected to Fourier transformation, the proportion of absorption to dispersion mode would vary across the spectrum. Fortunately this dephasing is to a good approximation linearly dependent on ΔH, and can thus be simply corrected with a first-order frequency-dependent phase angle after Fourier transformation.[6]

Consider then a train of equally spaced rf pulses with separation t sec. Let the magnetization components immediately before the pulse be M_z^-, M_x^-, and M_y^-. The critical assumption will be made that all transverse components of magnetization have been destroyed at this time (not simply dispersed by field inhomogeneity effects), so that $M_x^- = M_y^- = 0$. The justification for this assumption will be considered in more detail in Sec. II.B. During the pulse, a typical vector with an off-resonance parameter $\tan\phi$ will be nutated about the direction of H_{eff} through an angle ψ, and the three components of magnetization immediately after the pulse are readily shown to be

$$M_z^+ = (\sin^2\phi + \cos\psi\,\cos^2\phi)M_z^- = kM_z^-, \qquad (6)$$

$$M_x^+ = \sin\phi\,\cos\phi(1-\cos\psi)M_z^-, \qquad (7)$$

$$M_y^+ = \cos\phi\,\sin\psi M_z^-. \qquad (8)$$

(These are the equations used to calculate Fig. 1.) Consequently, the detected signal amplitude (without regard to phase) is given by

$$M_{xy}^+ = M_z^-\cos\phi[(1-\cos\psi)^2\sin^2\phi + \sin^2\psi]^{1/2} = \beta M_z^-. \qquad (9)$$

During the free precession period t the longitudinal component of magnetization will recover according to the equation

$$M_z{}^t = M_0[1 - \exp(-t/T_1)] + M_z^+\exp(-t/T_1)$$
$$= M_0[1 - \exp(-t/T_1)] + kM_z^-\exp(-t/T_1), \qquad (10)$$

where M_0 is the equilibrium magnetization, and the transverse components are assumed to have been destroyed. If a steady state is established with a dynamic balance between the effect of the pulses and spin–lattice relaxation, the quantities $M_z{}^t$ and M_z^- must be equal and hence from Eq. (10)

$$M_z^- = M_0[1 - \exp(-t/T_1)]/[1 - k\exp(-t/T_1)], \qquad (11)$$

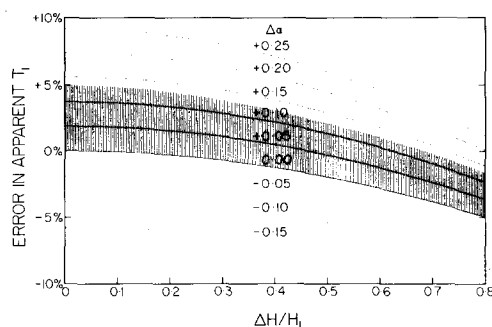$$M_{xy}^+ = \beta M_0[1 - \exp(-t/T_1)]/[1 - k\exp(-t/T_1)]. \qquad (12)$$

FIG. 2. In a progressive saturation experiment, the dependence of signal intensity $(S_\infty - S_t)$ on pulse interval t is expected to be exponential with time constant T_1. Imperfect pulses cause slight deviations from the ideal curve, introducing an error in the derived "apparent T_1". The percentage errors have been calculated as a function of the offset parameter $(\Delta H/H_1)$ for a family of curves representing small deviations $\Delta\alpha$ in the nominal flip angle from its ideal setting $\alpha = \pi/2$ rad. Operation within the shaded area (representing offsets ΔH up to $0.8H_1$, and deviations in flip angle $\Delta\alpha$ from 0 to 0.13 rad) will restrict the errors in the apparent relaxation time to $\pm 5\%$.

The parameter β is a function of the off-resonance parameter ϕ and the degree of missetting of the pulse angle α, but since it remains constant for a given line throughout a progressive saturation experiment, it affects all the intensity determinations equally and does not perturb the evaluation of T_1. However, the signal will only show a strictly exponential dependence on t if the parameter k is zero. It is apparent from Eq. (6) that this corresponds to the condition for zero longitudinal component of magnetization immediately after the pulse or, in a spectrometer with "ideal" rf pulses, the condition $\alpha = \pi/2$. There are therefore two crucial operating conditions which must be satisfied to ensure that the observed signals depend exponentially on t with time constant T_1,

(a) The magnetization before the pulse should be exclusively longitudinal $(M_{xy}^- = 0)$,

(b) The magnetization after the pulse should be exclusively transverse $(M_z^+ = 0)$.

For small values of k, the dependence on t will be close to exponential, and in practice the deviation from exponential shape will often not be detectable in the presence of statistical errors, although the apparent T_1 derived from a semilogarithmic plot will be in error. The problem is to establish the sensitivity to any deviation of the nominal flip angle α from $\pi/2$ radians, and to the off-resonance parameter $(\Delta H/H_1)$. The curve which represents Eq. (11) was evaluated numerically for suitable ranges of both these parameters, and an apparent spin–lattice relaxation time $(T_1{}^*)$ derived by fitting the results for M_z^- to the exponential $\exp(-t/T_1{}^*)$ at times $0.2T_1$ and $2T_1$. The choice of this time scale has some influence on the apparent T_1 (the later the measurements, the closer to the real T_1), but this range was judged to be closely representative of the one used in practice. Figure 2 illustrates the percentage errors in the apparent T_1 calculated in this way as a

function of $(\Delta H/H_1)$ and for a family of curves representing a range of values of the nominal flip angle α near $\pi/2$ rad. The first conclusion to be drawn is that quite large offsets $(\Delta H/H_1)$ can be tolerated without introducing an appreciable error. As might be anticipated, the errors for off-resonance spins can be reduced by deliberately setting the nominal flip angle α somewhat larger than $\pi/2$ radians, without introducing appreciable errors for components at or near resonance. The shaded area of Fig. 2 covers offsets ΔH up to 80% of H_1, and settings of the flip angle α between 90° and 97.5°, and yet restricts the maximum error to $\pm5\%$. In practice it is quite feasible to keep the experimental parameters well within these tolerances.

B. Transverse Components of Magnetization

The equations derived in Sec. II.A are based on the assumption that at the end of the free precession period transverse components of magnetization have been eliminated in such a way that they cannot be refocussed by later rf pulses. Clearly the amplitude of any such transverse components would be a function of the spin–spin relaxation times,[7] making it impossible to derive T_1 from a study of signal amplitudes versus t without some knowledge of the various spin–spin relaxation times.

Since it is well known that two or more 90° pulses will generate spin echoes of the type described by Hahn,[8] it is not sufficient to ensure that the spin isochromats are dispersed isotropically in the XY plane by static field inhomogeneity effects. Echo effects can be shown to be important for a train of *equally spaced* 90° pulses, not only in the transient mode soon after the pulses are first applied, but also in the steady-state that is eventually established. The details of the formation of such spin echoes have been treated theoretically and confirmed by experiment.[9] For proton or fluorine work the problem is further complicated by the modulation of echo amplitude due to homonuclear spin coupling.[10]

Alternative methods must therefore be devised to destroy all spin isochromats or to disperse them in a way that will not permit refocussing, or to scramble the echo amplitudes in such a way that when a large number of responses is accumulated the free induction decays reinforce but the echoes cancel. A field gradient pulse may be used to disperse the vectors isotropically in the XY plane,[4] and if a suitable sequence of *different* gradient pulses is applied, this should inhibit refocussing by the train of pulses.[11] Any internal field/frequency control loop would need to be protected from the momentary line broadening of the reference signal during the gradient pulses.

Another method of dispersing transverse magnetization in a nonreproducible manner would be to degrade the stability of the field/frequency control loop for a short time near the end of the precession period t, restoring the correct condition before the next rf pulse.

A noise pulse in the error signal circuit of the field/frequency regulator would accomplish this. The technique would become more effective the larger the number of transient signals subjected to time averaging.

Since the direction along which refocussing takes place depends on the relative phase of the rf field direction in the rotating frame from one 90° pulse to the next, incoherent rf pulses would cause a scrambling of echo amplitudes, leading to zero net echo amplitude after several responses have been accumulated. The rf pulses used in Fourier transform work are normally derived from a coherent source, but the randomization of the H_1 direction could be achieved by introducing a random delay in the timing of the rf pulse, easily achieved with computer-controlled pulsing. The delay would need to be sufficiently long that it induced a finite dephasing of the exciting radiofrequency with respect to the nuclear precession frequency closest to resonance (the slowest precession in the rotating frame). Since the desired free induction signals are generated by the effect of the rf pulse on *longitudinal* components of magnetization, they will always accumulate in phase.

In practice none of these proposed techniques proved to be necessary in the reported experiments on carbon-13
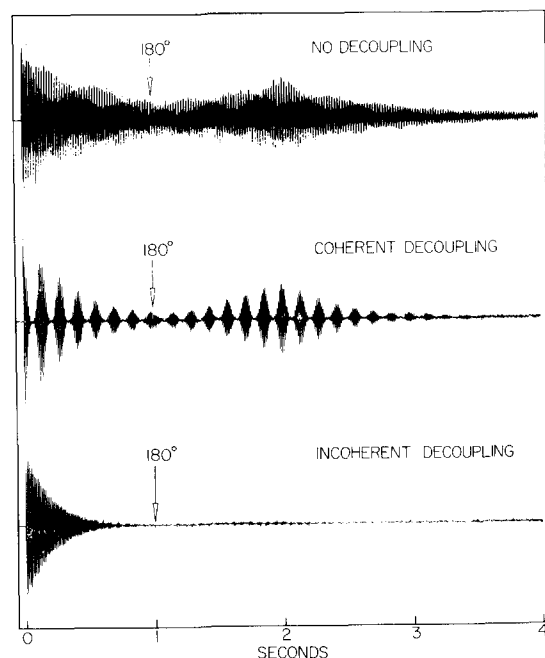

FIG. 3. The influence of heteronuclear decoupling on spin-echo formation. A 90°–180° pulse sequence has been applied to the carbon-13 resonance of an enriched sample of methyl iodide. Good echoes are observed with no decoupling (exhibiting a beat structure due to carbon–proton coupling), and with off-resonance coherent irradiation of the protons (showing beats due to the small residual carbon–proton splitting). However, with incoherent irradiation, all other parameters remaining unchanged, echo formation is greatly inhibited since the residual splitting becomes a random function of time, scrambling spin isochromats in a way which prevents refocussing. The two decoupled traces represent averages of 20 measurements each.

since it turned out that the proton noise decoupler[12] effectively destroyed transverse magnetization in a way that prevented refocussing. The reason for this was that the residual proton–carbon splittings act as a line broadening mechanism when the decoupling is incoherent, and in fact this was the dominant factor in determining the free induction decay in this case. The effect is demonstrated experimentally in Fig. 3 where spin echoes are excited in the carbon-13 resonance of enriched methyl iodide through the application of a 90°–180° pulse sequence. Good echoes are observed when the decoupler is off, or is set to the coherent mode, whereas only negligible refocussing takes place when the decoupler is set to incoherent operation.

C. Establishment of a Steady State

Significant errors in signal intensities are introduced if measurements are made before a steady-state response to the pulse train has been established, and this would lead to errors in the derived spin–lattice relaxation times. In order to analyze the approach to the steady state, it is sufficient to consider the behavior of the longitudinal magnetization only, assuming (as before) that there is no transverse component at the end of the free precession period. Since the first pulse is applied to a spin system in thermal equilibrium,

$$M_z^-(1) = M_0, \tag{13}$$

$$M_z^+(1) = kM_0, \tag{14}$$

$$M_z^t(1) = M_0(1-E_1) + kM_0E_1, \tag{15}$$

where E_1 has been written for $\exp(-t/T_1)$. The effect of a second pulse on this system can be written

$$M_z^+(2) = kM_z^t(1), \tag{16}$$

$$M_z^t(2) = M_0(1-E_1) + M_z^+(2)E_1$$
$$= M_0(1-E_1) + kM_0(1-E_1)E_1 + M_0(kE_1)^2. \tag{17}$$

By generalization, the effect of the nth pulse would be

$$M_z^+(n) = kM_z^t(n-1), \tag{18}$$

$$M_z^t(n) = M_0\{(1-E_1)[1+kE_1+\cdots(kE_1)^{n-1}]$$
$$+ (kE_1)^n\}. \tag{19}$$

As discussed in Sec. II.A, experimental conditions would normally be set up such that $k \leq 0.1$. Thus as $n \to \infty$, Eq. (19) reduces to

$$M_z^t(n) = M_0(1-E_1)/(1-kE_1). \tag{20}$$

This is just the steady-state solution of Eq. (11). At small values of n the errors in signal amplitude will be largest at short settings of t, that is when $E_1 \to 1$, when the last term of Eq. (19) may no longer be neglected with respect to earlier terms. Suppose that the measurements are restricted to conditions where t is never

shorter than $T_1/50$. (The practical requirements of resolution will normally require t to be of the order of one second or longer, so this assumption is quite justifiable in most experiments.) Then the minimum value of $(1-E_1)$ is 0.02, and with $k \leq 0.1$, it is possible to satisfy the condition for neglecting the last term,

$$(kE_1)^n \ll (1-E_1), \tag{21}$$

by choosing n equal to three. The corresponding approximation for longitudinal magnetization at the end of the third free precession period may then be written

$$M_z^t(3) \simeq M_0(1-E_1)[1+kE_1+(kE_1)^2], \tag{22}$$

which for small values of k is almost equivalent to Eq. (11). It is therefore concluded that a reasonably good approximation to the steady-state condition is attained after the first *three* free precession periods. Experiments were therefore set up to delay acquisition of free induction signals until at least three pulse cycles had elapsed.

III. EXPERIMENTAL

Natural abundance carbon-13 signals were recorded on a Varian XL-100 high resolution spectrometer operating at 25.1 MHz, and having a field/frequency control system based on the deuterium resonance signal, in the present case derived from a small quantity of benzene-d_6 added to the sample. The proton resonance region was irradiated from a 100-MHz source made incoherent over a band of several KHz by pseudorandom phase modulation to provide broad-band decoupling of all proton lines.[12,13] While the decoupler was operating, a constant flow of room temperature air through the probe maintained the sample temperature near 37°C. The sample was contained in an 8-mm-diam tube and was not degassed.

Radiofrequency pulses were generated by a Varian VFT-100 Fourier transform accessory that obtained timing commands from a small dedicated computer.[14] A 90° pulse had a duration of 55 μsec, corresponding to a pulse strength of 4.25 G. A fast analog-to-digital converter sampled up to 8192 points on each free induction decay, and typically 64 such transients would be accumulated to improve signal-to-noise. The transformation utilized the fast Fourier transform algorithm of Cooley and Tukey[15] as modified by Bergland.[16] In each measurement data acquisition was delayed until after the first 20 pulses of the train had occurred, in order to ensure that a steady state had been established. Since the spectrum studied divided naturally into two well-separated chemical shift regions, these were studied one at a time, the radiofrequency carrier being adjusted to each extreme of the spectrum in turn, and the unwanted spectral frequencies being attenuated by a suitable low-pass filter.[17] This ensures that the offset parameter $\Delta H/H_1$ is kept small for all components in the frequency range under investigation, so that the
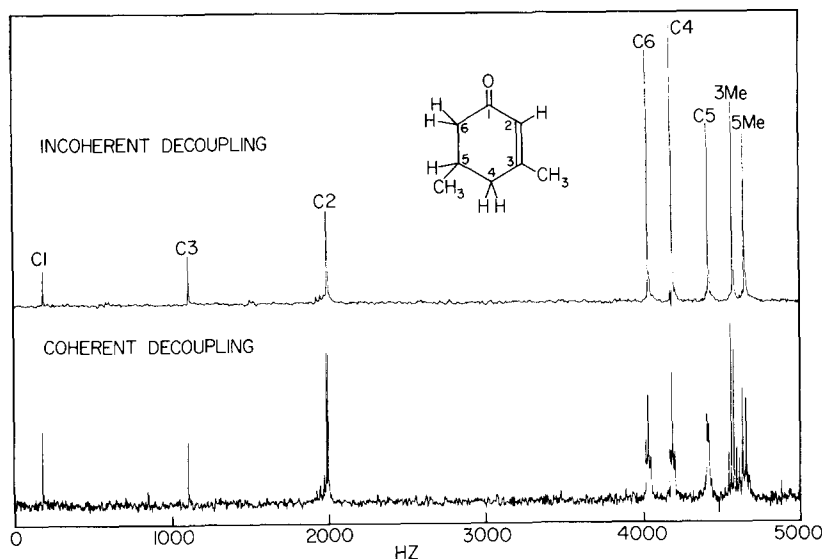
FIG. 4. Proton-decoupled carbon-13 Fourier transform spectra of 3,5-dimethylcyclohex-2-ene-1-one at 23 kG. Both traces represent 50 accumulations of the transient response. The lower trace, obtained with coherent off-resonance decoupling, exhibits the residual splitting into multiplets used to identify methyl, methylene, methine, and quaternary carbon atoms, part of the evidence used to make the assignment shown in the upper trace. The assignment of the methylene carbons C4 and C6 remains ambiguous.

parameter k is small, and longitudinal components after the pulse can be reasonably neglected. It also improves the fineness of digitization along the frequency axis, ensuring better reproduction of peak heights. After the transformation the computer stores the sine and cosine transforms, and uses them to calculate a spectrum for oscilloscope display, employing a phase angle and a frequency-dependent phase term entered by the operator.[6] In this way the phase parameters may be rapidly adjusted to give a pure absorption mode display across the entire spectrum, and the result is then plotted out on the spectrometer recorder.

As an experimental check on the proposed progressive saturation technique, spin–lattice relaxation measurements were also made on the same sample by the inversion-recovery method. The principle of the technique has been described elsewhere[4,5] but there was one significant practical improvement incorporated in the present experiments. Rather than record the instantaneous magnetization S_t and compare this with a measurement of the equilibrium magnetization S_∞ determined perhaps several hours later, the measurements were alternated and the two transient responses subtracted so that the deviation from equilibrium $(S_\infty - S_t)$ could be stored and later transformed. This was achieved through the pulse sequence:

$$[\cdots T \cdots 180° \cdots t \cdots 90°(S_t) \cdots T \cdots 90°(S_\infty)]_n,$$

where T is a time long compared with the longest T_1 to be measured. This greatly reduces the danger that slow drifts of spectrometer gain might introduce an error in the equilibrium measurement, to which all others are referred.

IV. RESULTS

All the relaxation measurements were carried out on a sample of 3,5-dimethylcyclohex-2-ene-1-one containing about 20% benzene-d_6 which provided the field/frequency locking signal. This sample was chosen because it contains examples of methyl, methylene, methine, and quaternary carbon atoms that would be expected to have significantly different spin–lattice relaxation times if dipole–dipole interaction with attached protons is a major factor.[18]

For reasons of simplicity the relaxation studies were performed on the noise-decoupled carbon-13 spectrum. However, the assignment of resonance lines was facilitated by recording undecoupled spectra and spectra observed with coherent off-resonance decoupling. Figure 4 illustrates how the off-resonance decoupling quickly identifies the carbon resonances in CH_3, CH_2, CH, and fully substituted C; together with the chemical shift
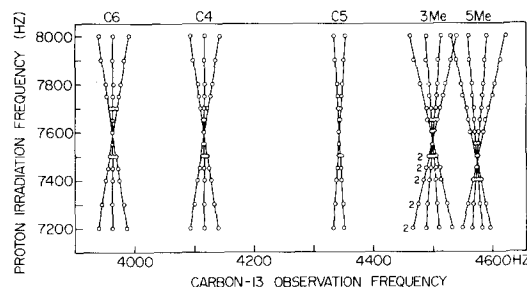

FIG. 5. A plot of the observed carbon-13 frequencies in the high-field region of the spectrum of 3,5-dimethylcyclohex-2-ene-1-one as a function of the (coherent) proton irradiation frequency (from an arbitrary zero). The markedly different coalescence points for the two methyl quartets clearly indicate that the high-field carbon is coupled to high-field protons, thus assigning it to position 5 in the ring. At low proton irradiation frequencies, the low-field carbon quartet exhibits an additional small splitting into a doublet (indicated by the numeral 2), presumably a long-range coupling to the proton at position 1, tending to confirm this assignment. Since there is no appreciable difference in coalescence point for the two methylene carbons (C4 and C6), their assignment remains ambiguous.

FIG. 6. The high-field region of the carbon-13 spectrum of 3,5-dimethyl-cyclohex-2-ene-1-one without proton decoupling. Note the complexity due to overlap of carbon–proton spin multiplets. The additional doubling of the low-field methyl quartet (~6 Hz) supports the assignment to the methyl carbon at position 3.
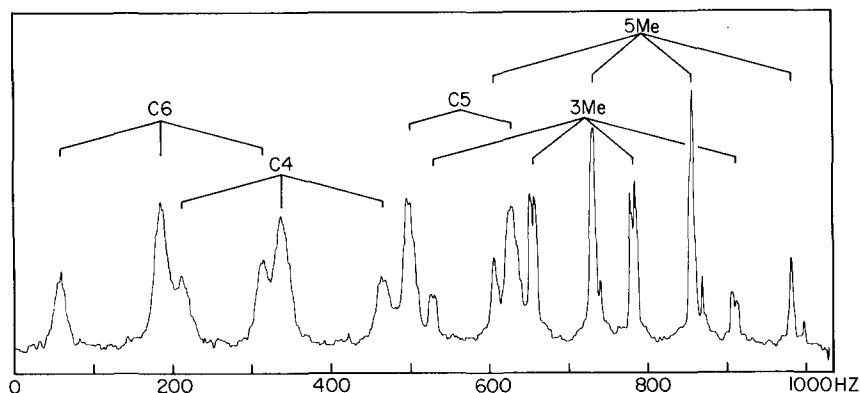
evidence this permits the assignment of the C1, C3, C2, and C5 resonances. This method avoids the complexity caused by overlap in the undecoupled spectrum. There remained ambiguities in the assignment of the two methylene resonances (C4 and C6) and the two methyl resonances (3Me and 5Me). A series of carbon-13 spectra were therefore recorded with coherent proton decoupling in order to correlate the shifts with the proton irradiation frequencies (see Fig. 5). Coalescence of the multiplet structure can be taken as an indirect indication of the corresponding proton shift. As it turned out, it was not possible to detect a difference between the methylene proton shifts large enough to be significant for assignment purposes. (Since the measured spin–lattice relaxation rates were also essentially indistinguishable, this proved to be of no practical importance.) In contrast, the two methyl quartets were found to coalesce at markedly different proton irradiation frequencies, indicating that the proton resonance corresponding to the low-field carbon occurs at higher frequency. The protons are thus on the olefinic methyl group (lower field), which identifies the carbon nucleus as 3Me rather than 5Me. At low proton irradia-

tion frequencies this carbon-13 quartet showed a further splitting into a doublet, attributed to coupling to the olefinic proton at C2, which is more easily decoupled at high proton irradiation frequencies. This long-range coupling was also detectable in the undecoupled spectrum of this region (Fig. 6). This evidence for the assignment of the methyl carbon nuclei is important because their spin–lattice relaxation rates were found to be markedly different.

A. Inversion–Recovery Measurements

To provide an independent measure of the carbon spin–lattice relaxation times in this sample, a series of determinations was made by the classic inversion–recovery method for comparison with the progressive saturation measurements. Since it can be shown that the inversion–recovery experiment is rather insensitive to the accuracy of the setting of the 180° and 90° pulse conditions, this would be expected to bring to light any inherent systematic errors of the progressive saturation method. The program is designed to minimize the effects of slow drifts of instrument gain, decoupling conditions, or resolution, by subtracting the transient

FIG. 7. Spin–lattice relaxation of carbon-13 nuclei in proton-decoupled 3,5-dimethylcyclohex-2-ene-1-one by the inversion–recovery technique. The spectrum was studied in two parts. The difference signal $(S_\infty - S_t)$ was obtained by subtraction of the transient responses observed with the pulse sequence $[T \cdots 180° \cdots t \cdots 90°(S_t) \cdots T \cdots 90°(S_\infty)]_n$, where $n = 64$ accumulations were made and where T was a time long compared with the longest T_1 in the frequency range under investigation. The experiment ran entirely under computer control, including pulse control, accumulation, Fourier transformation and the stacking of the records to create a three-dimensional impression. Note the scale change in the time axis of the low-field part of the spectrum necessitated by the very long relaxation times of C1 and C3.
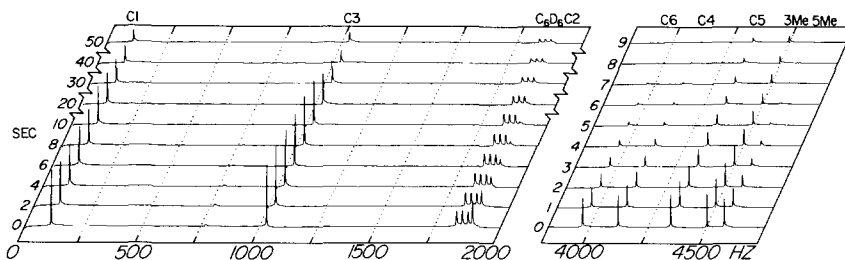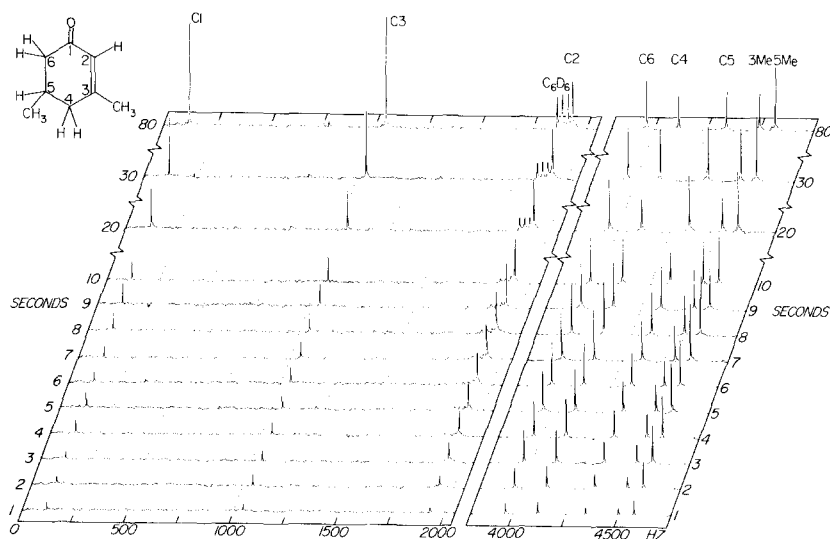
FIG. 8. Spin–lattice relaxation of carbon-13 nuclei in proton-decoupled 3,5-dimethylcyclohex-2-ene-1-one by the progressive saturation method. The spectra were obtained by Fourier transformation of 64 accumulated transient signals in the steady-state pulse regime for each setting of the pulse interval t seconds. With t set to 170 sec, a thermal equilibrium spectrum (not shown) was used to determine S_∞. For any given line, the quantity $(S_\infty - S_t)$ was found to be an exponential function of t, and the time constant was taken to be the spin–lattice relaxation time. Note the discontinuities in the time scale t occasioned by the need to follow the very slow relaxation of C1 and C3.

response S_t from the thermal equilibrium response S_∞, alternating the two measurements. A typical series of spectra is illustrated in Fig. 7; these spectra have been automatically offset by the computer in order to create a three-dimensional impression. Note that the two quaternary carbon nuclei (C1 and C3) and the benzene-d_6 solvent relax so much slower than the rest that a greatly extended time scale is required to accommodate them. The results (which represent averages of data not shown in Fig. 7) are the squares in the semilogarithmic plot of Fig. 9.

B. Progressive Saturation Measurements

The angle through which the magnetization is tipped by the radiofrequency pulse ($\alpha = \gamma H_1 \tau$ radians) was first set to the condition $\alpha = \pi$ by trial-and-error adjustment of the pulse width τ for minimum carbon-13 signal from an enriched sample of methyl iodide. For the relaxation measurements the pulse width was then halved, the computer-controlled timing maintaining high stability. The crossed-coil probe arrangement ensured good spatial homogeneity of the pulse over the effective volume of the receiver coil. The calculations of Sec. II.A, and in particular the estimates of the errors in the apparent T_1 illustrated in Fig. 2, indicate that offsets ΔH up to about $0.8H_1$ can be tolerated without seriously degrading the accuracy of the experiment. In practice the maximum offset never exceeded 2 kHz, because the spectrum was examined in two parts using a technique for recording partial Fourier transform spectra.[17] Thus the highest value of the offset parameter was 0.44, and if the errors predicted in Fig. 2 are representative, since no attempt was made to use a flip angle other than $\pi/2$, the maximum error from this source would be less than 2% (and in the negative direction). This was felt to be below the statistical errors of the measurements.

The time used for acquiring the transient data (which determines the attainable resolution of the experiment) also determines the minimum time between pulses, and in effect the shortest spin–lattice relaxation time that can be measured by this method. (This contrasts with the inversion–recovery technique where the critical time is the separation between the 180° and 90° pulses, which involves no data acquisition, and is limited only by the requirement for suppressing any Y component of magnetization excited by imperfections in the 180° pulse.) In the present series of experiments this acquisition time was typically 1 sec, which appeared to be adequate for the resolution of the carbon-13 spectra under investigation and long enough to permit transverse components of magnetization to be effectively dispersed before the next pulse. It should be possible to accommodate shorter times by adopting techniques which rapidly attenuate the net transverse magnetization while precluding refocussing by later pulses. One possibility is a deliberate reduction in the operating level of the proton noise decoupler after signal acquisition is complete but before the next pulse. Eventually a compromise must be struck between resolution and the shortest spin–lattice relaxation time to be measured.

A typical series of progressive saturation spectra of 3,5-dimethylcyclohex-2-ene-1-one is illustrated in Fig. 8. At each chosen value of the pulse interval a steady state was established by allowing the first 20 pulses to occur without acquiring data, and then 64 transient free induction signals were accumulated, subjected to Fourier transformation and the resulting spectrum recorded. The interval between pulses would then be increased by 1 sec, a new steady state established, and the next measurement started. As the pulse interval increases the signal peaks are seen to grow back towards the equilibrium value, a greatly extended time scale being required to follow the slowly relaxing C1 and C3

resonances and the benzene-d_6 solvent. A pulse interval of 170 sec was finally chosen as a sufficiently close approximation to the thermal equilibrium requirement, the effect of using too short a time being to underestimate the values of the long T_1's. Difference magnetizations $(S_\infty - S_t)$ were measured with respect to this spectrum since unlike the inversion–recovery technique, there is no way to measure this difference directly while still benefitting from the rapid accumulation of transient signals in the steady state.

Averaged data from several such experiments are compared directly with inversion–recovery results on the same sample in Fig. 9. The agreement is sufficiently good to permit both sets of results to be displayed on the same plot. A more searching test compares the spin–lattice relaxation times derived from the best fits to the experimental results plotted separately for the two techniques. Table I shows this comparison and it can be seen that the derived times never differ more than about 6%. Moreover, the differences appear to reflect the statistical errors of peak height determinations and there is no significant systematic trend between the two methods.

With a single notable exception, the spin–lattice relaxation times for this molecule show a definite inverse correlation with the number of directly attached protons, as would be expected if dipole–dipole interactions with protons are a major factor in the relaxation.[18] In fact the two methylene carbons, C4 and C6, have essentially indistinguishable relaxation times, while the small difference between the relaxation rates of the two methine carbons, C2 and C5, may not be significant either. Where there are no directly bound protons (C1 and C3) the difference in relaxation times would be

TABLE I. Comparison of the two experimental techniques for measuring spin–lattice relaxation times applied to 3,5-dimethylcyclohex-2-ene-1-one. Separate semilogarithmic plots were made of the experimental data obtained by the two different methods, and the corresponding time constants were derived independently.

Carbon nucleus	T_1 by progressive saturation (sec)	T_1 by inversion–recovery (sec)
C1	39	37
C3	32	33
3Me	6.0	5.9
C2	5.6	5.4
C5	5.4	5.3
C6	3.2	3.1
C4	3.3	3.1
5Me	2.6	2.7

expected to reflect longer-range influences such as the protons on neighboring carbon atoms; clearly C3, with the shorter relaxation time, has a preponderance of protons in its environment.

The methyl carbon at position 3 appears to be an exception, having a relaxation time more than twice that of the other methyl carbon. One possible explanation might lie in different correlation times for internal rotation, since the rotation of the methyl group at position 3 would appear to be less subject to steric hindrance.

V. DISCUSSION

The proposed technique for measuring spin–lattice relaxation times bears a close similarity to the progressive saturation method well known in continuous-wave magnetic resonance experiments. However, rather than follow signal intensities as a function of flip angle α at a constant setting of the pulse separation (which would represent a closer analogy with the continuous-wave experiment), the method employs a constant flip angle $(\alpha = \pi/2)$ and monitors the signal intensities as a function of pulse repetition rate. As a result there is a particularly simple relation between observed signal intensity and the spin–lattice relaxation time.

The experimental conditions must be arranged so that any *net* transverse magnetization is eliminated between pulses (not merely dispersed by the static field inhomogeneity, since then later pulses would refocus them to give spin echoes of the type described by Hahn[8]). This condition permits the pulse to act on longitudinal magnetization exclusively, with the result that spin–spin relaxation effects do not influence the time evolution of signal intensities. This is in marked contrast with the continuous-wave progressive saturation experiment which normally involves both relaxation times.

In the pulse-excited mode, "saturation" may be translated as the elimination of all longitudinal components of magnetization immediately after the pulse. This is the second required condition, and may be
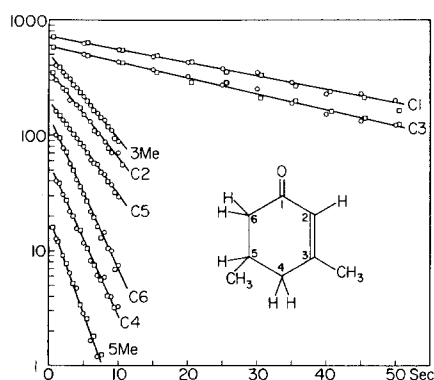

FIG. 9. Semilogarithmic plot of the inversion–recovery results (squares) and the progressive saturation results (circles) for the carbon-13 spin–lattice relaxation of 3,5-dimethylcyclohex-2-ene-1-one. The data represent means of several determinations, not simply the signals illustrated in Figs. 7 and 8. For clarity the inversion recovery results have been displaced horizontally to the right by the equivalent of one second (the relative vertical scaling is arbitrary). The fact that both sets of results can be resonably well fitted to the same straight lines is taken as evidence that the progressive saturation technique does not suffer from some serious systematic error.

violated either through poor adjustment of the flip angle α, or through the use of a radiofrequency field H_1 that is insufficiently strong in comparison with the offset ΔH of a given line from exact resonance. Residual longitudinal magnetization after the pulse results in a deviation from strictly exponential behavior and an error in the apparent spin–lattice relaxation time. Fortunately the experimental conditions are readily set up so as to contain such errors in the range of $\pm 2\%$. This adjustment of the flip angle α corresponds to the required calibration of the H_1 level in the continuous-wave progressive saturation experiment.

A theoretical analysis indicates that a steady-state balance between pulses and relaxation is rapidly established; only about three pulse cycles are normally required to reach conditions which differ by a negligible amount from the true steady state. When repeated accumulation of the transient signal is necessary for sensitivity reasons, there is no problem in arranging an initial period when pulses are applied but no signal sampling occurs. All the reported experiments involved this time-averaging feature because of the low intrinsic sensitivity achieved with carbon-13 resonances. For nuclei such as protons or fluorine, where acceptable signal-to-noise ratios may often be obtained from a single free precession transient, it might be feasible to establish a steady state once and for all at the beginning of the experiment, slowly reducing the pulse interval from very long times down to shorter times, and following the time evolution of S_t in the process.

Chemical interest focuses upon the application of this technique to the study of relaxation of individual components of high resolution spectra. The period t_0 over which the transient free induction signal is recorded determines the attainable resolving power of the experiment, $1/t_0$ Hz, and also sets the minimum interval between pulses, which determines the shortest spin-lattice relaxation time which can be effectively measured. Unless a noticeable degradation of the resolving power can be accepted, this normally restricts measured relaxation times to those of the order of one second or longer. Here the progressive saturation technique falls short of the inversion–recovery method, which is not bound by the same restriction since the interval between the π pulse and the $\pi/2$ pulse involves no data acquisition. However the spin-lattice relaxation times of the nuclei commonly studied by high resolution techniques tend to fall into the range above one second, and where relaxation is faster, a broadening of the slow-passage spectrum becomes perceptible.

At first sight the inversion–recovery experiment might be expected to exhibit better sensitivity than the progressive saturation technique, since by inverting spin populations rather than equalizing them it operates with a differential signal $(S_\infty - S_t)$ which is twice as intense. However this effect is compensated by the much more rapid accumulation of transient signals in the steady state mode that is established in the progressive saturation experiment, the inversion–recovery method being handicapped by the long period T which must be allowed for return to thermal equilibrium between each measurement. [If $T_1(\mathrm{max})$ is the longest spin–lattice relaxation time in the sample under investigation, then a reasonable value for the waiting period might be $5T_1(\mathrm{max})$.] The result is that when several measurements of S_t are made at regular intervals of t without prior knowledge of the trends of relaxation rates in the sample, both techniques exhibit a sensitivity of the same order.

However, the progressive saturation method does afford an operational flexibility denied to the inversion-recovery technique because, before the latter experiment can be started, the waiting period T must be set up and this requires an estimate of the longest relaxation time $T_1(\mathrm{max})$. Too short an estimate falsifies the measurement of S_t for the slowly relaxing nuclei (even at short times $t \ll T_1$), and to correct them the entire series must be repeated with a longer waiting period T. Too long an estimate reduces the sensitivity attainable in a given time, putting the technique at a disadvantage with respect to progressive saturation. In contrast, the progressive saturation experiment may proceed without any prior knowledge or estimate of $T_1(\mathrm{max})$, and whatever this turns out to be, all the measurements of S_t are valid. Both techniques involve a determination of S_∞, obtained by setting $t \approx 5T_1(\mathrm{max})$, estimated from the trend of S_t values as the experiment proceeds.

The present treatment of spin–lattice relaxation measurements has deliberately set out to minimize possible systematic errors with a view to obtaining results that may be relied on to within a few percent. An entirely different approach may be considered. The experiment lends itself very well to the determination of the order of magnitude of T_1, and in particular to the still simpler problem of deciding which of two resonance lines has the longer T_1. This last piece of information may be all that is needed to make an assignment of the two lines. Once the spectrum has been obtained by Fourier transformation of transient signals recorded at one pulse repetition rate, all that may be necessary is a redetermination at a second pulse repetition rate, not necessarily one corresponding to thermal equilibrium. (Such a technique may rival the off-resonance coherent decoupling method of assigning carbon-13 lines according to the number of directly attached protons.) For this kind of determination the sensitivity of the progressive saturation method exceeds that of the inversion-recovery technique—often a very useful economy of instrument time.

Two slightly different operational modes have come to be accepted in Fourier transform spectroscopy. The first[3] employs a fixed pulse repetition rate determined by the resolution requirements, and optimizes sensitivity by adjusting the flip angle α. If there are several

different spin–lattice relaxation rates in the spectrum, this setting must be a compromise. Usually in practice, unless the relaxation times are short with respect to the pulse interval, the optimum flip angle is considerably less than $\pi/2$ rad. The second mode[19] utilizes a constant flip angle $\alpha = \pi/2$ rad and optimizes sensitivity by setting the pulse interval to the condition $t/T_1 = 1.269$. Again this adjustment must be a compromise if there are several different relaxation rates. It has been calculated[19] that this second mode of operation achieves an optimum sensitivity only 81.4% of that obtained in the first mode. The proposed progressive saturation technique provides spin–lattice relaxation data that is a useful aid to both these adjustments. However it is related in a particularly direct manner to the second mode of operation. In fact, in cases where the 20% loss in sensitivity can be tolerated, there is much to be said for operating with $\alpha = \pi/2$ and variable pulse interval, since by repetition of the experiment at a second pulse interval it is a simple matter to extract qualitative information about the spin–lattice relaxation times. There may be other practical advantages of this second mode of operation that are related to the relatively long waiting period between the completion of signal acquisition and the next rf pulse. In practice this time interval might be of the order of 10 sec, and could therefore be used to prepare the spin system in some particular way, for example, a redistribution of spin populations through the internuclear Overhauser effect by noise irradiation of some coupled nucleus, the irradiation field being extinguished just before the next pulse in order to eliminate the decoupling effect in the transformed spectrum.[20,21] Since most Fourier transform spectrometers include a laboratory computer, this could be set to work during the waiting period on additional calculations or control functions. For example the time interval is of the right order to permit calculation of the Fourier transform *after each transient*, instead of a single transformation after a large number of transients have been accumulated. This would permit monitoring of any time-dependent features in the spectrum, including the progress of the signal-to-noise ratio as the accumulation proceeds.

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