

Measurement of the Relaxation Rates of ^1H Longitudinal Modes

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Accurate decay rate measurements for longitudinal modes are essential for many of the methods proposed for investigating molecular dynamics by NMR. However, the effects of cross relaxation often make it impossible to determine accurate values for these quantities. A method is presented that enables the effects of cross relaxation to be largely eliminated from such measurements. Its reliability is assessed by comparing the values for internuclear distances that can be determined from the resulting relaxation rates with values obtained using other methods. Other consequences of using this technique include an increased robustness of experiments to short (i.e., $<5T_1$) relaxation times and the ability to make multiple "selective" relaxation measurements simultaneously. © 1997 Academic Press

INTRODUCTION

NMR relaxation measurements are an established tool for probing molecular dynamics (1–6). Typically, a number of relaxation rates dependent upon different linear combinations of the spectral density function are measured. This information can be analyzed in two ways: in terms of the correlation times and order parameters associated with molecular motion (7, 8) or to determine values for the spectral density function at specific frequencies (9). The latter approach has the advantage that it makes no assumptions about the motion of the molecule and is independent of any particular model.

While it is sometimes useful to measure the relaxation rates of individual transitions, it is becoming more common, particularly in studies of biomolecules, to measure the relaxation rates of specific longitudinal modes or in-phase and antiphase components of coherence. This latter approach has the advantage that it does not require individual multiplet transitions to be resolved from each other; its main disadvantage is that it requires that the particular process whose rate is to be measured be isolated from all other processes. This presents problems because individual processes cannot usually be considered in isolation: the relaxation of a set of interacting spins can only be described accurately by a set of coupled differential equations that include all other possible longitudinal modes, or components of coherence, that may arise from the system, as appropriate.

The effects of cross relaxation can be ameliorated to an

extent in some measurements, such as those of longitudinal magnetization, by ensuring that only the species of interest is perturbed from equilibrium (10). This allows the assumption to be made that, initially at least, the decay of the species of interest is unaffected by cross relaxation. However, the perturbation of other species from equilibrium is often an unavoidable side effect of exciting the species of interest. This is usually true for all spin orders between two or more spins, where the longitudinal magnetization of one or more of the spins involved in the mode will be perturbed from equilibrium during its excitation. For nuclei such as ^1H , where chemical-shift anisotropy is small, this does not usually affect the decay of longitudinal two-spin order since it does not cross relax with longitudinal magnetization as a result of purely dipolar relaxation. However, for three-spin order, where only dipolar interactions are required for cross relaxation with longitudinal magnetization to occur, the effects may be substantial.

Techniques for suppressing cross-relaxation effects in heteronuclear measurements of both transverse and longitudinal relaxation rates are well established (9, 11), and homonuclear experiments that utilize the same principles to suppress cross relaxation in the measurement of ^1H transverse magnetization, longitudinal magnetization, and longitudinal two-spin order have been proposed (12–16). The approaches used include the application of continuous irradiation or a sequence of selective 180° pulses to a band in the spectrum to suppress spin diffusion to that region (9, 11, 12), synchronous nutation of a pair of spins to suppress spin diffusion to other spins (13), and the use of one or more selective 180° pulses to suppress one or more selected cross-relaxation pathways (14–16).

Here we investigate the suppression of cross-relaxation effects in decay rate measurements of homonuclear ^1H longitudinal modes. The basic types of cross-relaxation process and the mechanisms by which they can occur are considered, and this information is used to ascertain their relative importance in both slow- and fast-tumbling regimes. The principles used to suppress cross-relaxation effects, and their implementation for measuring the decay rates of arbitrary longitudinal modes, are discussed. These principles can be used to design experiments which are tolerant to imperfect inversions and short recovery times ($<5T_1$) and can be used to

perform multiple ‘‘selective’’ measurements simultaneously. The reliability of the relaxation rates obtained and their use for determining the internuclear distances between coupled spins is also considered.

CROSS RELAXATION

In systems consisting of protons or other nuclear species for which chemical-shift anisotropy can be considered to make a negligible contribution to relaxation, two types of cross-relaxation process can occur between longitudinal modes. A pair of spins can be exchanged to interconvert a pair of modes of the same order with all but one spin in common. This type of process is summarized by

$$I_{iz} \prod_s 2I_{sz} \leftrightarrow I_{jz} \prod_s 2I_{sz}, \quad [1]$$

where i and j are the spins which are exchanged and the spins s are common to the two modes. The rate for a process of this type is given by

$$\begin{aligned} R(I_{iz} \prod_s 2I_{sz} \leftrightarrow I_{jz} \prod_s 2I_{sz}) \\ = 0.5 D_{ij} D_{ij} [(-1/3)J(\omega_i - \omega_j) + 2J(\omega_i + \omega_j)] \\ + \sum_s 0.5 D_{si} D_{sj} [2J(\omega_s)] (3 \cos^2 \theta_{si,sj} - 1)/2, \quad [2] \end{aligned}$$

where $D_{ab} = -(\mu_0/4\pi)(6\pi/5)^{1/2} \gamma_a \gamma_b \hbar / r_{ab}^3$ and $\theta_{ab,ac}$ is the angle between the internuclear vectors \mathbf{r}_{ab} and \mathbf{r}_{ac} . Cross relaxation can also interconvert modes differing by two in the total number of their spins, where all but these two spins are common to the two modes

$$(1/2) \prod_s 2I_{sz} \leftrightarrow 2I_{iz} I_{jz} \prod_s 2I_{sz}. \quad [3]$$

The rate for a process of this type is given by

$$\begin{aligned} R[(1/2) \prod_s 2I_{sz} \leftrightarrow 2I_{iz} I_{jz} \prod_s 2I_{sz}] \\ = \sum_s 0.5 D_{si} D_{sj} [2J(\omega_s)] (3 \cos^2 \theta_{si,sj} - 1)/2. \quad [4] \end{aligned}$$

The relative importance of these two types of process will clearly depend on the geometry and motional properties of the spins concerned. For a process of the type given in Eq. [3] to occur, a spin active in the mode must be close to at least two others, while for a process of the type given by Eq. [1] to occur, an active spin need only be near one other. Consequently, processes of the type given by Eq. [1] are likely to occur most frequently. Since the processes represented by Eq. [1] effectively depend on $J(0)$ in a homonuclear spin system, they can be expected to remain significant in both slow- and fast-tumbling regimes, while those represented by Eq. [3] depend only on $J(\omega_s)$ and consequently

may only be expected to be effective in the fast-tumbling regime.

Simulations of the ratio of cross- to auto-relaxation rates as a function of the rotational correlation time for the component of longitudinal magnetization I_{az} of a spin a with dipolar interactions with two other spins b and c at equidistance on either side of it are given in Fig. 1. The model-free spectral density function given by

$$\begin{aligned} J(\omega) = (1/4\pi) \{ S^2 [2\tau_c / (1 + \omega^2 \tau_c^2)] \\ + (1 - S^2) [2\tau / (1 + \omega^2 \tau^2)] \} \quad [5] \end{aligned}$$

is assumed. Here, $1/\tau = 1/\tau_c + 1/\tau_i$, where τ_c is the overall rotational correlation time, τ_i is the correlation time for internal motion and S^2 is an order parameter. Simulations are given for a rigid molecule ($S^2 = 1$), represented by bold lines, and for one with significant internal motion ($S^2 = 0.5$ and $\tau_i = 50$ ps), represented by narrow lines. Continuous lines give the ratio of cross- to auto-relaxation rates when no measures are taken to suppress cross relaxation. The component of longitudinal magnetization I_{az} can cross relax with I_{bz} and I_{cz} with rates given by Eq. [2], and with $4I_{az}I_{bz}I_{cz}$ with a rate given by Eq. [4]. Since some of procedures suggested for suppressing cross-relaxation effects remove only the former (14), the ratio obtained under these conditions is given by dashed lines. While both types of cross-relaxation processes have positive rates in the fast-tumbling regime, the former becomes negative in the slow-tumbling regime as $J(\omega_i - \omega_j)$ becomes dominant. This causes the ratio to become negative, passing through zero at one point.

Suppressing cross relaxation between modes on the same order accounts for less than three quarters of the overall cross-relaxation rate in the fast-tumbling regime, which is clearly inadequate if accurate auto-relaxation rates are to be measured. As the slow-tumbling regime is entered, the importance of the unsuppressed processes rapidly decreases, although relatively fast internal motion can reduce the rate of decline. However, even in this latter case, the ratio is reduced to 0.1 when $\tau_c = 2 \times 10^{-9}$ s and 0.02 when $\tau_c = 1 \times 10^{-8}$ s. It is interesting to note that, in the transitional region between the two regimes, suppressing cross relaxation between modes on the same order alone can actually *increase* the magnitude of the relaxation-rate ratio.

SUPPRESSION OF CROSS-RELAXATION EFFECTS

In the context of designing suppression procedures for cross-relaxation effects, it is useful to differentiate between direct and indirect cross-relaxation processes. Cross relaxation can be classified as direct when it occurs as a direct consequence of another species being prepared with a non-equilibrium amplitude. For example, in a nonselective inversion-recovery experiment, the amplitude of I_{az} may be perturbed as a result of the direct cross-relaxation process I_{bz}

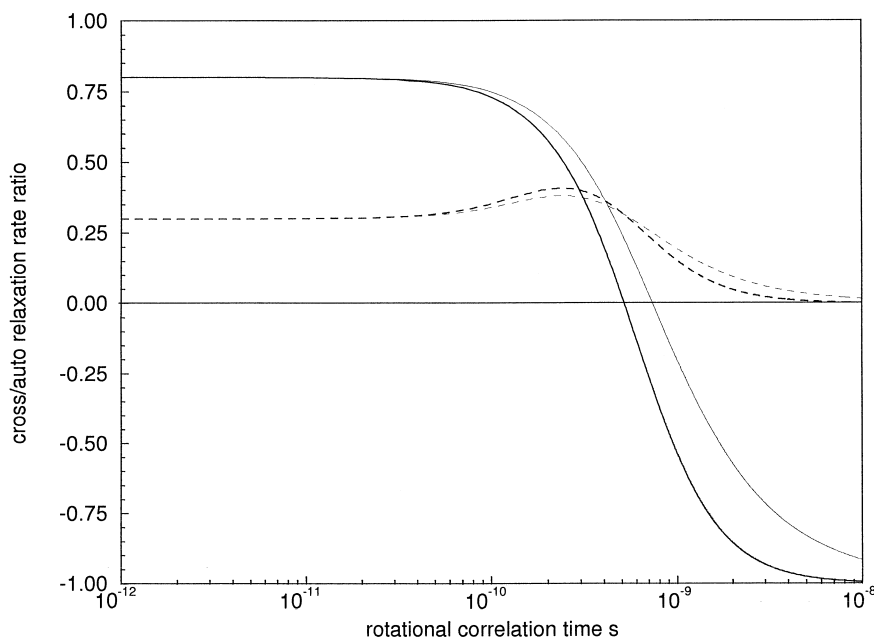


FIG. 1. Variation of the ratio of cross/auto dipolar relaxation rates with rotational correlation time for the longitudinal magnetization of the central spin of an equidistant linear three-spin system. Solid lines include all cross-relaxation processes while dashed lines include only cross relaxation with longitudinal three-spin order. Two cases are considered: without internal motion (bold lines) and with internal motion with $S^2 = 0.5$ and $\tau_i = 50$ ps (thin lines).

$\rightarrow I_{az}$ from I_{bz} . Indirect cross relaxation occurs as a result of the species of interest being initially perturbed from equilibrium: cross relaxation from it perturbs other species which in turn, through cross relaxation, perturb the amplitude of the species initially selected. For example, in the inversion-recovery experiment described above, the indirect cross-relaxation process $I_{az} \rightarrow I_{bz} \rightarrow I_{az}$ may alter the amplitude of I_{az} . Unlike direct cross-relaxation processes, indirect processes will occur in both selective and nonselective experiments, since these processes depend only upon the amplitude of the species of interest being initially perturbed from equilibrium. In general, direct cross-relaxation processes have the greatest effect on relaxation measurements when present, but neither direct nor indirect processes can be ignored if the results are to be used quantitatively.

At least two species are involved in any cross-relaxation process. It has been shown that cross relaxation can be suppressed by changing the sign of one of these species halfway through a period of time (9, 11–16). While cross relaxation still occurs during both intervals, the sign of any change in amplitude of either species that it results in is reversed, resulting in mutual cancellation of the effect over the whole time period. The less the overall amplitudes of the two species concerned have changed over this period of time, the more effectively cross-relaxation effects will be suppressed. The method of choice for changing the sign of a homonuclear longitudinal mode is to apply 180° semi-selective pulse(s) to an odd number of its active spins.

The type of process outlined in Eq. [1], which involves

exchanges between pairs of modes of the same order, usually makes the largest contribution to cross relaxation in both slow- and fast-tumbling regimes. The effects of cross-relaxation processes of this type can be suppressed by inverting all of the spins active in the mode of interest. In the case of a component of longitudinal magnetization I_{az} , applying a $180^\circ(a)$ pulse will clearly change the sign of I_{az} but not of other components of longitudinal magnetization. In the case of a component of longitudinal two-spin order $2I_{az}I_{bz}$, applying a $180^\circ(a, b)$ pulse will *not* affect the sign of the chosen mode, but *will* invert all those components of longitudinal two-spin order with which it can cross relax, for example, $2I_{az}I_{cz}$, since they will only have one spin in common with it. For a component of longitudinal three-spin order $4I_{az}I_{bz}I_{cz}$, a $180^\circ(a, b, c)$ pulse will invert the chosen mode but not those of other components of three-spin order with which they can exchange since they will have only two spins in common. The sign of one of a given pair of modes can of course be changed by inverting a single spin that is not common to both, but this would usually seem to be a less preferable option since it suppresses only a subset of those processes described by Eq. [1].

The effects of cross-relaxation processes of the type given in Eq. [3] cannot all be removed by inverting a single set of spins. Instead, it is necessary to consider each process individually and to invert a spin not common to the two species concerned. In practice, suppressing cross-relaxation effects arising from a number of processes of the types given in Eqs. [1] and [3] requires implementing a number of inde-

pendent suppression procedures in the same experiment. This can be achieved by interleaving them during the mixing period τ .

A single suppression procedure comprising the inversion of a set of spins i can be implemented by applying the inversion at the center of the mixing period:

$$\tau/2—180^\circ(i)—\tau/2. \quad [6]$$

An additional suppression procedure comprising the inversion of a set of spins j can be interleaved with it by making the suppression time (i.e., the time after which a suppression procedure has been completed) of one procedure a power of two times longer than that of the other. In practice, this means that one procedure must be repeated at least twice as often as the other:

$$\tau/4—180^\circ(i)—\tau/4—180^\circ(j)—\tau/4—180^\circ(i)—\tau/4. \quad [7]$$

This sequence can be readily extended to incorporate a third suppression procedure comprising the inversion of spins k :

$$\begin{aligned} &\tau/8—180^\circ(i)—\tau/8—180^\circ(j)—\tau/8—180^\circ(i) \\ &\quad—\tau/8—180^\circ(k)—\tau/8—180^\circ(i)—\tau/8 \\ &\quad—180^\circ(j)—\tau/8—180^\circ(i)—\tau/8. \quad [8] \end{aligned}$$

Further suppression procedures can be included in a similar manner. In practice, the length of the overall procedure may be determined by the signal loss arising from each set of inversions, the minimum length of the mixing period imposed by the length of the soft pulses, and if phase cycling is used to select coherence transfer pathways, the length of any phase cycle needed to remove any coherence excited by the inversion pulses. When several procedures are interleaved, the most effective overall suppression will be obtained by suppressing most frequently those cross-relaxation processes which have the greatest effect on the amplitude of the species of interest. The dominant processes will usually be those which depend on the smallest internuclear distances according to Eqs. [2] and [4].

MEASUREMENT OF LONGITUDINAL MAGNETIZATION

Pulse sequences for measuring relaxation usually consist of three basic parts, a preparation period during which the species to be investigated is perturbed from equilibrium, a mixing period during which it is allowed to relax, and a detection period during which it is converted into observable magnetization and subsequently detected. The detection period may incorporate a filter to prevent unwanted species from being observed.

A selective inversion-recovery pulse sequence for measur-

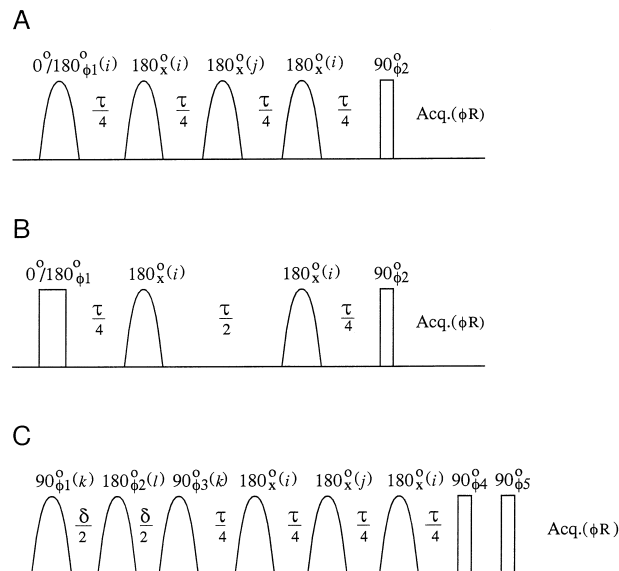


FIG. 2. Pulses sequences for measuring the relaxation of (A) the longitudinal magnetization of spin i with the cross-relaxation-suppression scheme given in Eq. [7], (B) multiple components of longitudinal magnetization with the cross-relaxation-suppression scheme given in Eq. [9], and (C) longitudinal spin order between spins l with the cross-relaxation-suppression scheme given in Eq. [7]. For (C), the longitudinal mode is prepared from one of its active spins k with scalar couplings to all of the others; $\delta = 1/(2J)$. Phase cycling for (A) and (B): $\phi_1 = (x, -x)$, $\phi_2 = 2(x, -x)$, $\phi_R = 2(x, -x)$. Data acquired with both flip angles of the initial pulse are subtracted from each other. Phase cycling for (C) when longitudinal two-spin order is to be measured: $\phi_1 = x$, $\phi_2 = 8(x, y, -x, -y)$, $\phi_3 = 4(y, -y)$, $\phi_4 = 45^\circ$, $\phi_5 = (x, y, -x, -y)$, $\phi_R = (x, -y, -x, y) + 4(x, -x) + 8(x, -x)$; if a nonselective 180° pulse is used in the preparation period, the phase cycle can be reduced to 16 steps by using $\phi_1 = 8(x, -x)$ and $\phi_2 = x$. When longitudinal three-spin order is to be measured: $\phi_1 = x$, $\phi_2 = 12(x, y, -x, -y)$, $\phi_3 = 6(x, -x)$, $\phi_4 = (0^\circ, 60^\circ, 120^\circ, 180^\circ, 240^\circ, 300^\circ)$, $\phi_5 = 30^\circ$, $\phi_R = (x, -x, x, -x, x, -x) + 6(x, -x) + 12(x, -x)$; if a nonselective 180° pulse is used in the preparation period, the phase cycle can be reduced to 24 steps by using $\phi_1 = 12(x, -x)$ and $\phi_2 = x$. For all of these experiments, all other pulses in the mixing period τ can also be phase cycled through 180° while keeping the receiver phase constant. The number before each bracketed cycle indicates the number of consecutive transients that are acquired with each step. Where a phase expression is a linear combination of bracketed cycles, the phases calculated for each one are added together to obtain the phase to be used.

ing the decay of longitudinal magnetization is given in Fig. 2A. The mixing period incorporates selective $180^\circ(i)$ pulses to suppress cross relaxation to other components of longitudinal magnetization and a selective $180^\circ(j)$ pulse to suppress cross relaxation with longitudinal three-spin order. The experiment is performed with the initial pulse set to both 180° and 0° , and the results are subtracted from each other to compensate for the fact that the equilibrium amplitude of longitudinal magnetization is nonzero (14–16). Providing that it is accurately calibrated, the 90° detection pulse will convert any components of spin order arising from cross relaxation exclusively into multiple-quantum coherence, which will not be observed.

The effects of various inversion-recovery experiments on

a component of longitudinal magnetization I_{1z} arising from a system consisting of spins 1–3 undergoing dipolar relaxation in both fast- and slow-tumbling regimes are simulated in Fig. 3. As might be expected, in both cases the nonselective inversion-recovery experiment is most strongly affected by cross relaxation, giving decay curves furthest from the ideal. However, in the fast-tumbling regime, cross relaxation causes the magnetization to decay more quickly than the ideal while in slow-tumbling, cross relaxation causes it to decay more slowly. This is due to a change of sign in the cross-relaxation rate given by Eq. [2] arising from the dominance of $J(\omega_i - \omega_j)$ in the slow-tumbling regime. This change of sign affects only the sign of direct cross-relaxation effects; indirect cross-relaxation effects are expected to decrease the decay rate in both regimes. The conventional selective inversion-recovery experiment produces data that initially decays at a rate close to the ideal but that rapidly decreases thereafter.

In the fast-tumbling regime, inverting spin 1 with the mixing period scheme given in Eq. [6] to remove cross relaxation with other components of longitudinal magnetization produces better agreement. Inverting spin three in addition, according to the mixing-period scheme given in Eq. [7], to remove cross relaxation to longitudinal three-spin order produces data that effectively coincide with the ideal down to 40% of the initial signal intensity; this can be extended to the whole simulation by repeating this mixing sequence. In the slow-tumbling regime, it is only necessary to invert spin 1 for the simulated data to coincide with the ideal down to 50% of the initial intensity since only cross relaxation with modes on the same order is significant. Repeating the procedure produces good agreement to the end of the simulation.

It has been assumed above that all pulses used for cross-relaxation suppression behave ideally. In reality, this is unlikely to be the case. The effects of suppression sequences comprising 120 instead of 180° pulses are simulated in Fig. 4A. All other conditions are the same as for Fig. 3B. The three decay curves are scaled to the same initial intensity to facilitate comparison. The incomplete inversion of 1 resulting from the use of a single 120°(1) pulse clearly greatly reduces the effectiveness of cross-relaxation suppression. However, a train of two 120°(1) pulses, i.e., nominally

$$\tau/4—180^\circ(i)—\tau/2—180^\circ(i)—\tau/4, \quad [9]$$

returns the effectiveness of suppression to levels comparable with that demonstrated in Fig. 3B. The inclusion of a third pulse reduces the effectiveness of the suppression of cross-relaxation effects. This suggests that even numbers of pulses are self-compensating for this type of nonideal behavior and that the error in signal amplitude arising from the nonideal behavior of one pulse can undergo mutual cancellation with that arising from the next. The effects of nonideal behavior

are likely to be most serious for modes with the largest amplitudes since incomplete inversion will result in greatest absolute loss of intensity.

In the suppression sequence given in Eq. [7], the two 180°(*i*) pulses are self-compensating but the single 180°(*j*) pulse is not. This problem may be overcome by repeating the sequence. Alternatively, any consequences of nonideal behavior of the 180°(*j*) pulse can be minimized by using it to invert modes with the smallest amplitudes or to suppress only relatively unimportant cross-relaxation pathways. Similarly, the suppression sequence given in Eq. [8] is self-compensating for the 180°(*i*) and 180°(*j*) pulses but not for 180°(*k*).

A suppression sequence will affect not only the amplitude of the species of interest perturbed from equilibrium during the preparation period, but also those species not initially perturbed with which it undergoes cross relaxation. Simulations of the perturbation of spin 2 from equilibrium as a result of cross relaxation from 1 are given in Fig. 4B for various suppression sequences. When the pulses are assumed to behave ideally, i.e., to produce 180° rotations, a mixing time incorporating two suppression sequences suppresses the net transfer $I_{1z} \rightarrow I_{2z}$ approximately seven times more effectively than a single pulse; the addition of a third pulse makes little difference. For the current simulations, when a single 180°(1) pulse is used, the amplitude of magnetization transferred to I_{2z} can be up to 7% of the initial intensity of I_{1z} , while when two or three pulses are used, this is reduced to less than 1%. When 120°(1) pulses are used, the amplitude of magnetization transferred from I_{1z} to I_{2z} increases. The application of a two 120°(1) pulse sequence suppresses this effect approximately three times more effectively than a single pulse; using three 120°(1) pulses improves suppression by a further third.

The incorporation of cross-relaxation-suppression procedures into the mixing period has the side effect of making the pulse sequence more robust than usual toward short recovery periods between experiments. Traditionally, a recovery period of at least $5T_1$ was regarded as necessary between experiments for measuring longitudinal modes. This allows all modes to return to equilibrium before the next experiment starts; if they have not done so, cross relaxation from them may distort the measurement being made. However, if the mixing period is constructed in such a way that cross-relaxation effects are efficiently suppressed, this consideration no longer applies, and in principle, much shorter recovery periods may be used. In practice, it should be borne in mind that cross-relaxation suppression is unlikely to be completely effective.

A further consequence of suppressing the effects of cross relaxation between modes is that multiple selective measurements can in principle be made in the same experiment. An experiment of this type is given in Fig. 2B. Here the initial selective pulse of Fig. 1A has been made nonselective so that all spins are inverted. In terms of cross relaxation, the

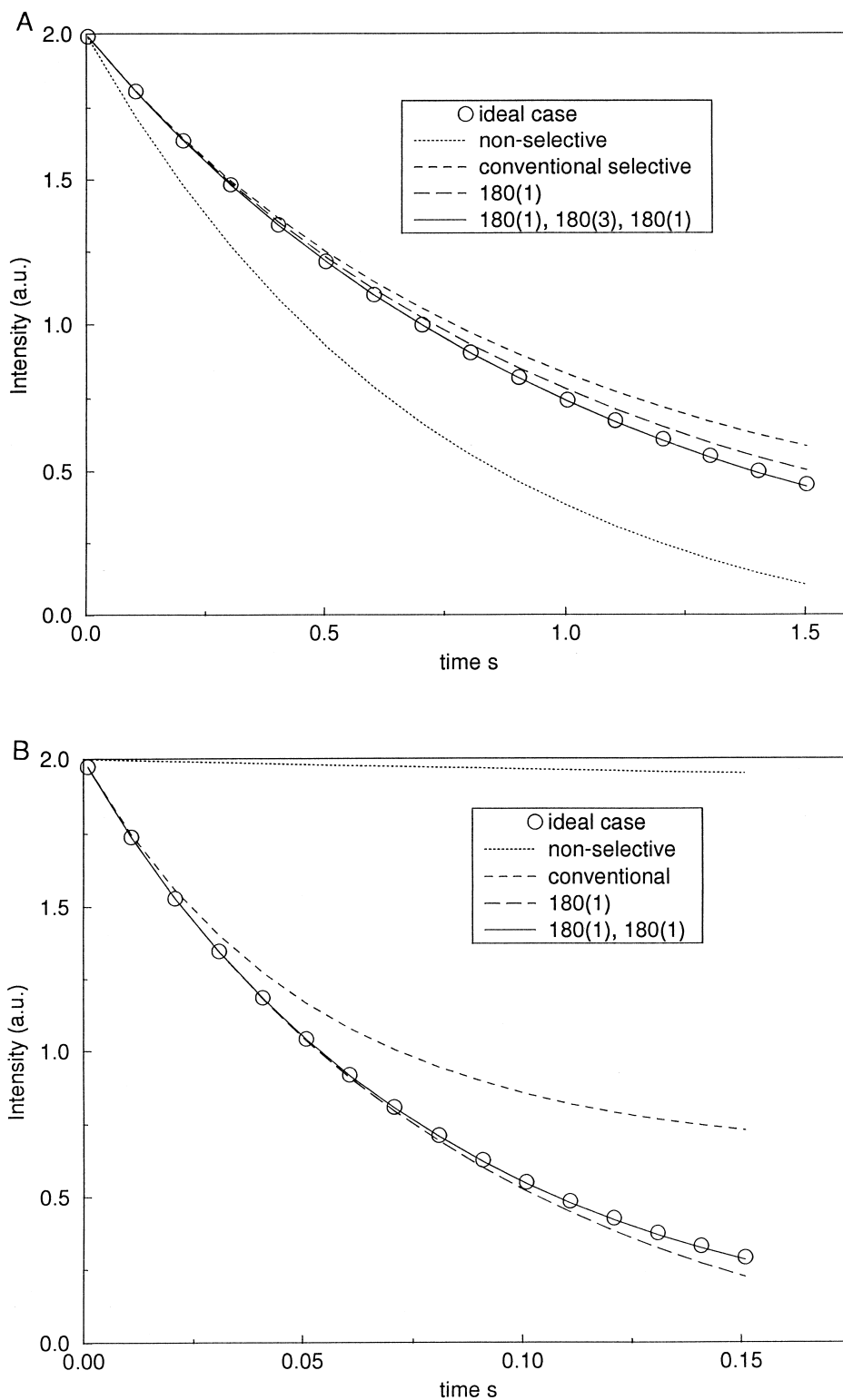


FIG. 3. Simulations of inversion-recovery relaxation data for I_{1z} arising from a three-spin system in (A) the fast-tumbling regime and (B) the slow-tumbling regime. The designation 180(1) corresponds to the inversion of spin 1 according to the scheme given in Eq. [6], while 180(1), 180(1) corresponds to the scheme given in Eq. [6] repeated twice and 180(1), 180(2), 180(1) refers to the inversion of 1 and 2 according to the scheme given in Eq. [7]. The magnitudes of all intensities, which are to the same vertical scale, are given for ease of comparison.

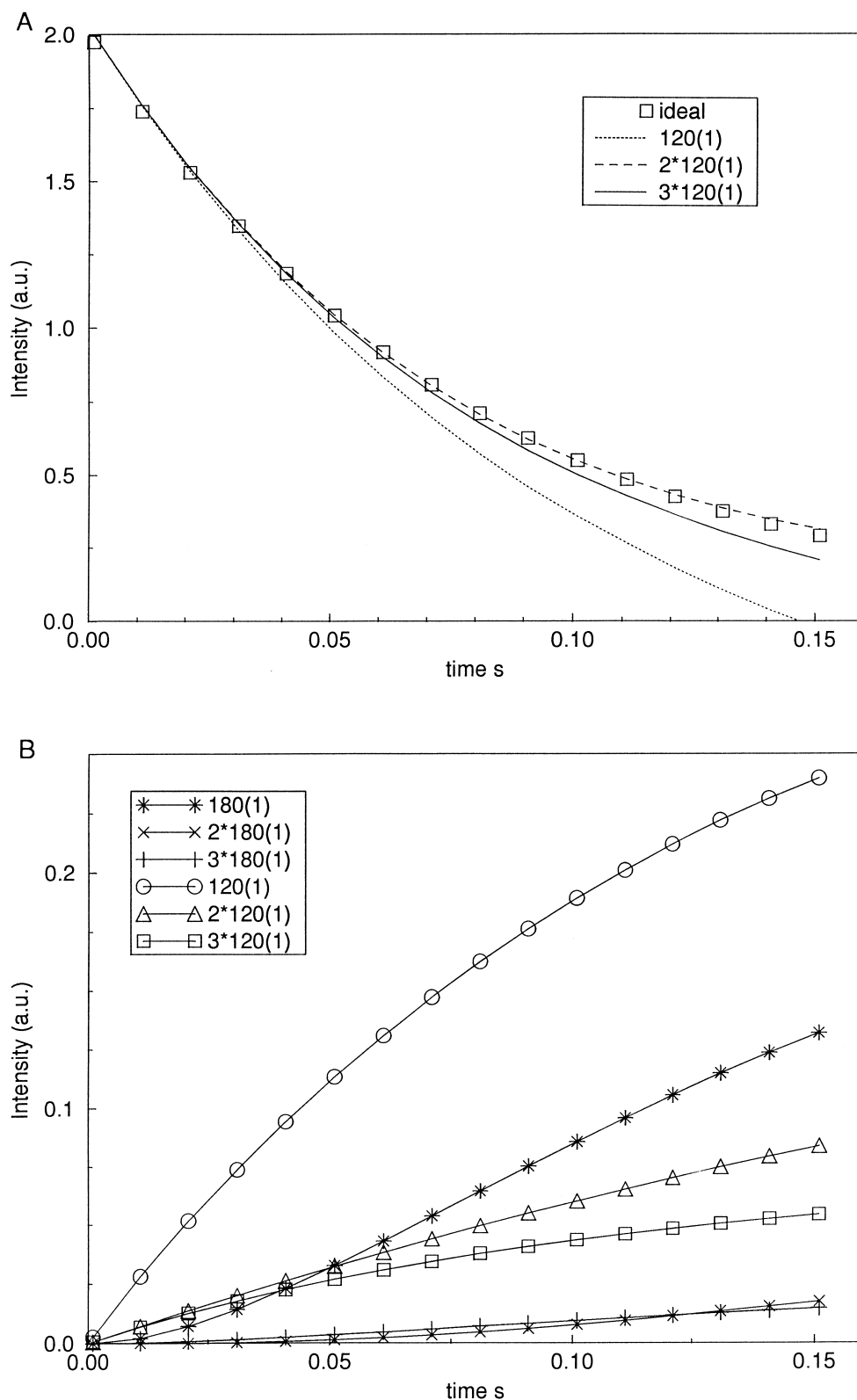


FIG. 4. (A) Simulations of the decay of I_z under the same conditions as in Fig. 3B except that all inversion pulses produce an effective angle of rotation of 120° . $n * 120(1)$ denotes a mixing period corresponding to Eq. [6] repeated n times with 120° (instead of 180°) rotations applied to spin 1. The magnitudes of the decay curves are scaled to the same initial intensity for ease of comparison. (B) Simulations of the perturbation of I_z from equilibrium arising from selective-inversion-recovery measurements of I_z under the same conditions as in Fig. 3B. The cross-relaxation-suppression schemes used are as indicated in the figure. All simulations are to the same scale, which is that of Fig. 3.

main difference between this and the equivalent selective experiment is that direct as well as indirect processes must now be considered. Any effects on the species of interest arising from direct cross relaxation will be significantly larger than those arising from indirect processes and consequently should be given priority in suppression. Since practical considerations are likely to limit the number of suppression procedures implemented in a single experiment, this approach is likely to be most effective if the components of longitudinal magnetization chosen have their largest dipolar interactions with each other. This means that each suppression procedure will benefit at least two of the chosen components of longitudinal magnetization.

The effects of this experiment on a component of longitudinal magnetization I_{1z} arising from a two-spin system are simulated in Fig. 4. In Fig. 4A, simulations are given for three ratios of the longitudinal relaxation rates of the two spins, and data simulated for a conventional nonselective inversion recovery experiment are given for comparison. When $R(I_{1z}) = R(I_{2z})$, this represents a special case since inverting spin 1 using the mixing period scheme given in Eq. [6] produces data that *exactly* coincides with the ideal. The reason for this can be seen by considering the pair of coupled differential equations that describe longitudinal relaxation for this system

$$-d[I_{1z}(t)]/dt = R(I_{1z})\Delta I_{1z} + R(I_{1z} \leftrightarrow I_{2z})\Delta I_{2z} \quad [10a]$$

$$-d[I_{2z}(t)]/dt = R(I_{2z})\Delta I_{2z} + R(I_{1z} \leftrightarrow I_{2z})\Delta I_{1z}, \quad [10b]$$

where ΔI_{1z} and ΔI_{2z} correspond to the difference between the current and equilibrium amplitudes of the two components of longitudinal magnetization. Since $R(I_{1z}) = R(I_{2z})$, $\Delta I_{1z} = \Delta I_{2z}$ after the initial inversion, and consequently Eq. [10] can be rewritten as

$$-d[I_i(t)]/dt = [R(I_{1z}) + R(I_{1z} \leftrightarrow I_{2z})]\Delta I_i, \quad [11]$$

where $i = 1$ or 2 . From Eq. [11], it can be seen that during the first half of the mixing period the amplitudes of both spins will decrease exponentially with the rate $[R(I_{1z}) + R(I_{1z} \leftrightarrow I_{2z})]$. The inversion of spin 1 at the center of the mixing period will change the sign of the second term on the right of Eq. [11]; as a consequence of this, both spins will decay exponentially with the rate $[R(I_{1z}) - R(I_{1z} \leftrightarrow I_{2z})]$ during the second half of the mixing period. The overall rate of decay will therefore clearly be $R(I_{1z})$.

The further the ratio of $R(I_{1z})/R(I_{2z})$ deviates from 1.0, the less effectively cross-relaxation effects will be suppressed. In these circumstances, the effectiveness of suppression can be increased by repeating the mixing sequence, as can be seen in Fig. 5B. As might be expected from Fig. 4B, using two inversion pulses (Eq. [9]) substantially increases the effectiveness of suppression. The addition of a third pulse results in only a marginal further improvement.

MEASUREMENT OF LONGITUDINAL SPIN ORDER

Pulse sequences for measuring the relaxation of longitudinal spin order have the same basic structure as those for measuring longitudinal magnetization (17–19). However, while the latter can be designed to perturb only the chosen component of longitudinal magnetization from equilibrium, Fig. 2A, in the case of the former, Fig. 2C, perturbation of modes from equilibrium other than the chosen one is unavoidable. This has two main consequences: direct cross relaxation may occur between the perturbed modes during the mixing period, and care must be taken in determining from which mode the detected magnetization originates.

In the case of longitudinal two-spin order, if a selective 180° pulse is applied to the two active spins alone at the center of the preparation period, only the chosen mode and the longitudinal magnetization of its participating spins will be perturbed from equilibrium. Since chemical-shift anisotropy is not usually thought to be significant for protons, this does not present a problem since cross relaxation will not occur between them. If the 180° pulse in the mixing period is nonselective, all possible modes between the initially excited spin and its scalar-coupling partners may be generated, allowing multiple selective measurements to be made in a manner analogous to that for longitudinal magnetization. The same considerations for suppressing cross relaxation between them apply as for longitudinal magnetization. The detection period includes a double-quantum filter to remove any unwanted longitudinal magnetization. If several components of longitudinal two-spin order have been excited, they should be measured on the spins they *do not* have in common as all will contribute to the signal observed for this spin.

In the case of longitudinal three-spin order, if a selective 180° pulse is used in the preparation period, only the chosen mode and the longitudinal magnetization of its constituent spins will be perturbed from equilibrium. However, unlike the case for longitudinal two-spin order, these modes can cross relax with each other, making direct cross-relaxation inevitable and the first target for suppression procedures. Inverting any two of the three-spin-order participating spins according to the mixing-period scheme given in Eq. [7] suppresses these processes together with any indirect cross-relaxation processes with other components of three-spin order between which the inverted spins are not common participants.

Higher-spin orders can be dealt with in a similar manner. In general, if p -spin order is being prepared, $(p - 2n)$ spin orders, where n is a positive integer such that $n > p/2$, will also be perturbed from equilibrium. Longitudinal magnetization is always perturbed from equilibrium.

EXPERIMENTAL

Simulations given in Figs. 3–5 were calculated numerically. For Figs. 3 and 4, the full dipolar-relaxation matrix

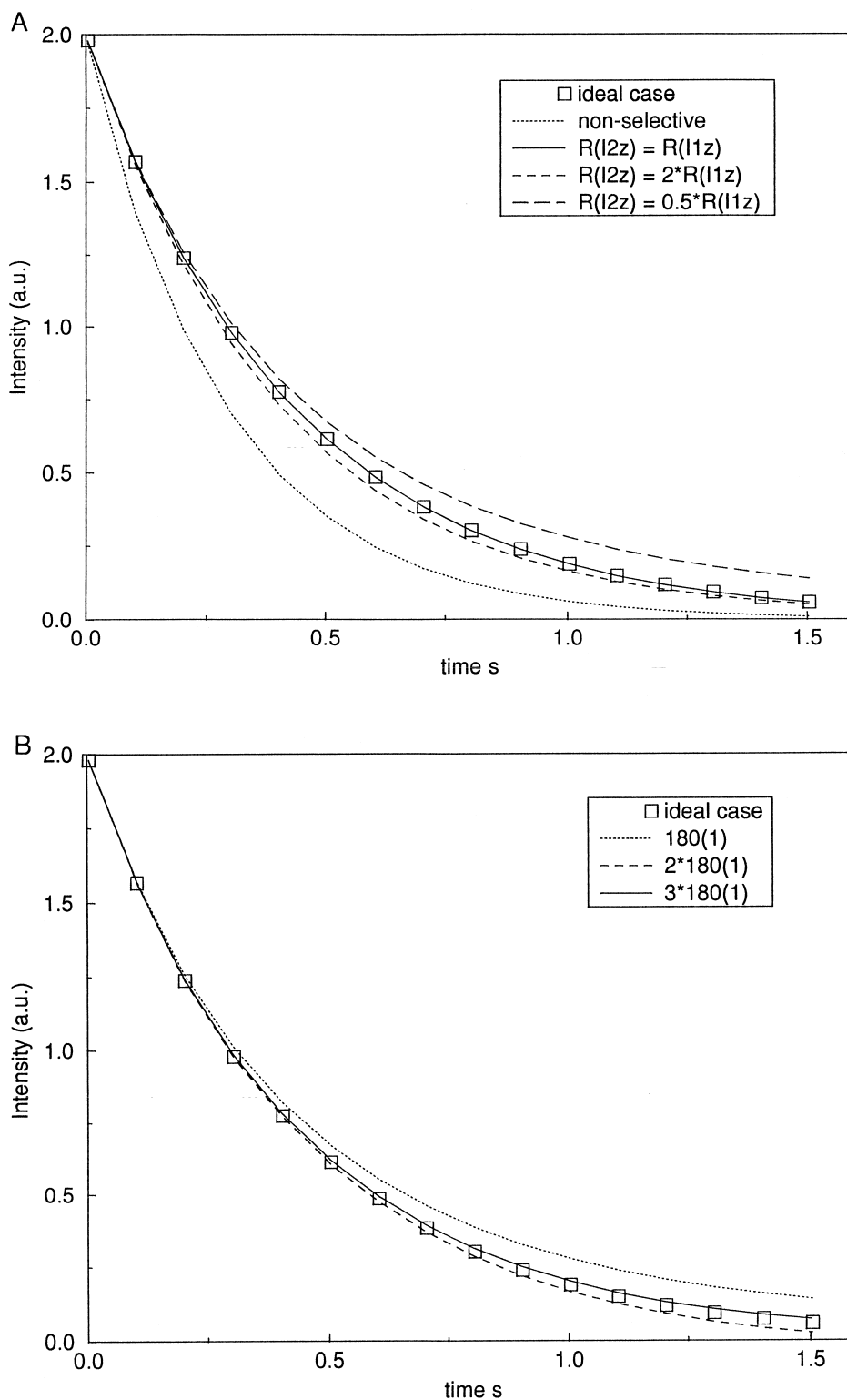


FIG. 5. Simulations of the decay of I_{1z} arising from a two-spin system in a nonselective-inversion-recovery experiment with (A) the cross-relaxation-suppression sequence given in Eq. [6] applied to spin 1 and (B) the cross-relaxation-suppression procedures indicated for the case $R(I_{2z}) = 0.5 \cdot R(I_{1z})$.

was used for a set of three spins, denoted 1–3, with relative coordinates of (0.0, 0.0, 0.0), (0.0, 0.0, 2.0), and (0.0, 0.0, -1.9) Å, respectively. For Fig. 3A, $\tau_c = 5 \times 10^{-11}$ s, while

for Figs. 3B and 4, $\tau_c = 6 \times 10^{-9}$ s. For Fig. 5, two spins 1.5 Å apart denoted 1 and 2 were used. The full dipolar-relaxation matrix calculated for $\tau_c = 5 \times 10^{-11}$ s was used

in each case except for $R(I_{2z})$ which was set as indicated in the figure.

All experiments were performed either at 298 K on a Bruker ARX-250 NMR spectrometer operating at 250 MHz for ^1H or at 300 K on a Bruker DRX-400 operating at 400 MHz for ^1H as indicated in the figure legends.

Experiments on allyl bromide were performed on a degassed and sealed 0.1 M solution in CDCl_3 . Measurements of longitudinal magnetization were performed using the pulse sequence given in Fig. 2A with the suppression sequence given in Eq. [7] repeated twice (unless otherwise indicated), where i is the spin being measured and j is its nearest neighbor. The two equivalent H4 spins were omitted from this procedure.

Measurements of longitudinal two-spin order were performed using the pulse sequence given in Fig. 2C, except that a nonselective 180° pulse was used in the preparation period in the cases of $2I_{1z}I_{3z}$ and $2I_{2z}I_{3z}$, with the suppression sequence given in Eq. [8], where i and j are its active spins and k is the remaining spin out of H1–H3. $2I_{1z}I_{2z}$ was prepared from H1 using $\delta = 432$ ms, $2I_{1z}I_{3z}$ was prepared from H1 using $\delta = 18$ ms, and $2I_{2z}I_{3z}$ was prepared from H2 using $\delta = 38$ ms.

The measurement of longitudinal three-spin order was performed using the pulse sequence given in Fig. 2C with the suppression sequence given in Eq. [7], where $i = \text{H1}$ and $j = \text{H3}$. $4I_{1z}I_{2z}I_{3z}$ was prepared from H3 using $\delta = 40$ ms.

A 30 ms Gaussian waveform was used for all selective 90° pulses. A 50 ms q3 Gaussian cascade was used for all selective 180° inversion pulses applied to H3, and a 60 ms cascade was used for all selective 180° inversion pulses applied to H1 and H2. A minimum 2 s delay was left between inversion pulses in the mixing period to allow any unwanted coherence excited to decay. The mixing time increment used was 0.9 s in all cases. The number of transients acquired for each mixing time was 2 for longitudinal magnetization, 16 for longitudinal two-spin order, and 48 for longitudinal three-spin order. A relaxation delay of 140 s was used in all cases except for longitudinal three-spin order, where 100 s was used, unless otherwise stated.

Experiments on sucrose octaacetate were performed on a sealed and degassed 0.1 M solution in 1:1 $\text{C}_6\text{D}_6/\text{CDCl}_3$. Measurements of longitudinal magnetization were performed using the pulse sequence given in Fig. 2A with the suppression sequence given in Eq. [7], where i is the spin being measured and j is a near neighbor. For H1, $j = \text{H2}$; for H2, $j = \text{H4}$; for H3, $j = \text{H5}$; and for H4, $j = \text{H2}$.

Measurements of longitudinal two-spin order were performed using the pulse sequence given in Fig. 2C, except that a nonselective 180° pulse was used in the preparation period. The suppression sequence given in Eq. [7] was used, where i and j are the active spins except for $2I_{4z}I_{5z}$, where H4 and H3 were used since the H5 multiplet overlaps with other resonances. $2I_{1z}I_{2z}$ was prepared from H1 using $\delta = 92$ ms, $2I_{2z}I_{3z}$ was prepared from H2 using $\delta = 40$ ms, $2I_{3z}I_{4z}$

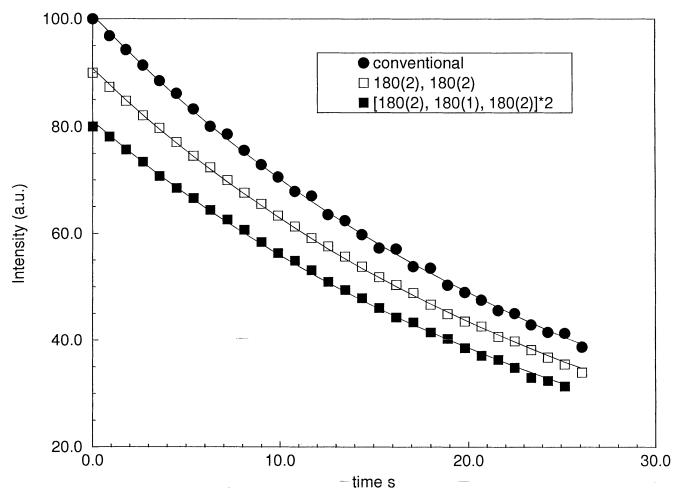


FIG. 6. The 400 MHz ^1H relaxation data for I_{2z} of 0.1 M allyl bromide in CDCl_3 .

was prepared from H4 using $\delta = 16$ ms, and $2I_{4z}I_{5z}$ was prepared from H4 using $\delta = 16$ ms.

Measurements of longitudinal three-spin order were performed using the pulse sequence given in Fig. 2C, except that a nonselective 180° pulse was used in the preparation period, with the suppression sequence given in Eq. [7], where i and j are two of its active spins. For $4I_{1z}I_{2z}I_{3z}$, i and j were H2 and H3, for $2I_{2z}I_{3z}I_{4z}$, i and j were H3 and H4, while for $2I_{3z}I_{4z}I_{5z}$, i and j were H4 and H3. $4I_{1z}I_{2z}I_{3z}$ was prepared from H2 using $\delta = 38$ ms, $2I_{2z}I_{3z}I_{4z}$ was prepared from H3 using $\delta = 40$ ms, and $2I_{3z}I_{4z}I_{5z}$ was prepared from H4 using $\delta = 40$ ms.

A 30 ms Gaussian waveform was used for all selective 90° pulses, and a 50 ms q3 Gaussian cascade was used for all selective 180° pulses. Magnetic-field-gradient pulses of 4 ms lengths were applied after all RF pulses in the mixing period to dephase any unwanted coherence excited. A balanced pair of 4 ms magnetic-field-gradient pulses were also applied around 180° pulses in the preparation period, where applicable, instead of phase cycling. The mixing time increments used were 32 ms for longitudinal magnetization, 24 ms for longitudinal two-spin order, and 16 ms for longitudinal three-spin order. The number of transients acquired for each mixing time was 1 for longitudinal magnetization, 16 for longitudinal two-spin order, and 24 for longitudinal three-spin order. A relaxation delay of 15 s was used in all cases.

RESULTS AND DISCUSSION

Relaxation data for I_{2z} of allyl bromide is given in Fig. 6. Data acquired with the conventional selective inversion-recovery experiment yielded a relaxation rate of $3.62 \pm 0.02 \times 10^{-2} \text{ s}^{-1}$. Suppression of the effects of indirect cross-relaxation processes with other components of longitudinal

relaxation according to the scheme given in Eq. [9] increased this to $3.68 \pm 0.02 \times 10^{-2} \text{ s}^{-1}$. The additional suppression of cross relaxation with longitudinal three-spin order by inverting H1 as well as H2 using the scheme given in Eq. [7] further increased this to $3.71 \pm 0.02 \times 10^{-2} \text{ s}^{-1}$. The suppression scheme given in Eq. [7] was repeated twice in this latter case to take advantage of the self-compensating properties with respect to nonideal behavior of even numbers of inversion pulses noted above. The inversion of H3 instead of H1 in this latter case to suppress cross-relaxation effects with longitudinal three-spin order would be expected to yield the same result since H1 and H3 are the nearest spins to H2; this procedure (data not given) yielded a rate within 0.4% of that obtained by inverting H1, and within experimental error. The relaxation rates given above are all within 3% of each other; larger variations in the rates given above would be expected if data were measured over a greater range of signal attenuations.

Relaxation data for all of the longitudinal modes arising from H1, H2, and H3 of allyl bromide are given in Fig. 7A. All components of longitudinal magnetization were measured using the mixing period given in Eq. [7] (repeated twice) to suppress the effects of cross relaxation to other components of longitudinal magnetization and to longitudinal three-spin order. Components of longitudinal two-spin order were measured using the mixing period given in Eq. [8] to suppress the effects of cross relaxation with other two-spin modes and four-spin modes incorporating the spin k . For ease of implementation, the former was achieved by inverting the active spins i and j separately instead of together; in principle, both procedures should yield the same results. This procedure was used throughout the current work for both two-, and three-spin modes. Longitudinal three-spin order was measured using the mixing sequence given in Eq. [7] to suppress the effects of cross relaxation with all components of longitudinal magnetization and other three-spin modes which do not have either i or j in common.

To test their quality, linear combinations of the relaxation rates were taken to isolate the mutual relaxation of pairs of nuclear spins using (23)

$$R(I_{az}) + R(I_{bz}) - R(2I_{az}I_{bz}) \\ = \frac{1}{2}D_{ab}^2[(2/3)J(\omega_a - \omega_b) + 4J(\omega_a + \omega_b)]. \quad [12]$$

$$R(2I_{az}I_{cz}) + R(2I_{bz}I_{cz}) - R(I_{cz}) - R(4I_{az}I_{bz}I_{cz}) \\ = + \frac{1}{2}D_{ab}^2[(2/3)J(\omega_a - \omega_b) + 4J(\omega_a + \omega_b)]. \quad [13]$$

This procedure eliminates all contributions to dipolar relaxation other than those between a and b , in each case. Unlike the alternative test of comparing the results with calculated values for the relevant elements of the relaxation matrix, this method is not susceptible to errors arising from interactions with other molecules or paramagnetic species in solution.

Since the fragment of allyl bromide containing H1–H3 is rigid, the ratio of Eq. [12] or [13] for all three pairs of protons should simply be the ratio of the inverse sixth powers of their internuclear distances. The ratio of the inverse sixth powers of the internuclear distances of H1–H2:H1–H3:H2–H3 is 29.8:1.5:6.6. Using Eq. [12], the mutual relaxation rates of H1–H2, H1–H3, and H2–H3 are calculated to be 0.0283(5), $-0.0001(5)$, and $0.0096(5) \text{ s}^{-1}$, respectively, a ratio of 28.3:–0.1:9.6. Using Eq. [13], the corresponding results are 0.0261(17), $-0.0022(17)$, and $0.0075(17) \text{ s}^{-1}$, respectively, which corresponds to a ratio of 29.4:–2.2:8.5. The greater error in the latter three rates reflects the relatively large error in the decay rate of the longitudinal three-spin order.

A comparison of the rates obtained using Eqs. [12] and [13] with the theoretical ratio of their values reveals a difference which cannot be accounted for solely by experimental error. It is likely that this discrepancy arises from cross relaxation with modes incorporating the two H4 spins, the effects of which cannot be suppressed since it is not possible to invert one H4 spin without inverting the other. For example, when the decay of I_{1z} is being measured, it is not possible to suppress the effects of its cross relaxation with $4I_{1z}I_{4z}I_{4z}$. The differences between the rates obtained using Eqs. [12] and [13] probably reflect the differing effects of unsuppressed cross-relaxation pathways on the rates using in the calculations.

Relaxation data for some of the one-, two-, and three-spin longitudinal modes arising from sucrose octaacetate are given in Fig. 7B. Due to the relatively fast rates of relaxation of the modes arising from this molecule compared to the time required to perform a selective inversion, suppression sequences were limited in length to three pulses (Eq. [7]), resulting in a minimum mixing-period length of 150 ms. The inclusion of an additional suppression procedure (Eq. [8]) would have increased the minimum mixing period length to 350 ms, by which time many of the two- and three-spin modes measured would have decayed by up to 50% of their initial amplitudes.

For measuring the decay of a given component of longitudinal magnetization, the effects of cross relaxation to other components of longitudinal magnetization were suppressed by inverting the spin of interest. The effects of cross relaxation to three-spin modes are suppressed by also inverting its nearest neighbor in space. The spin of interest is inverted twice (i in Eq. [7]) to take advantage of the self-compensating properties associated with even numbers of inversions for suppressing the effects of what are likely to be the dominant cross-relaxation processes.

For longitudinal two-spin order, the two active spins in each mode were inverted according to the scheme given in Eq. [7] to suppress the effects of cross relaxation to other two-spin modes. The effects of cross relaxation with longitudinal magnetization, which may occur if chemical-shift anisotropy is significant, are also suppressed by this sequence.

In the case of longitudinal three-spin order, two of the

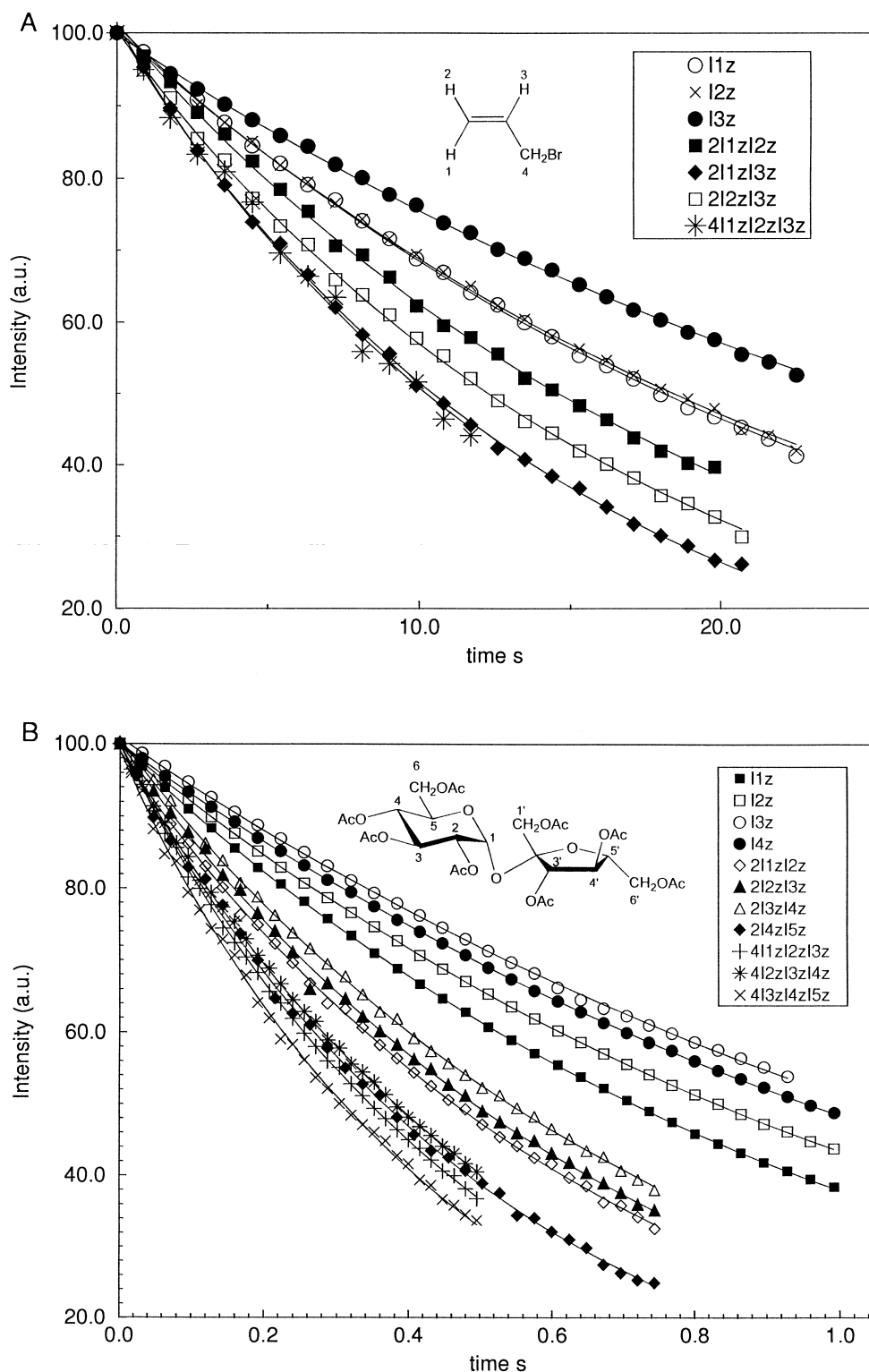


FIG. 7. The 400 MHz ^1H longitudinal-mode relaxation data for (A) 0.1 M allyl bromide in CDCl_3 and (B) 0.1 M sucrose octaacetate in 1:1 $\text{C}_6\text{D}_6/\text{CDCl}_3$. The relaxation rates calculated for allyl bromide from the data in (A), assuming exponential decay, are $R(I_{1z}) = 0.0388 \pm 0.0002 \text{ s}^{-1}$, $R(I_{2z}) = 0.0379 \pm 0.0002 \text{ s}^{-1}$, $R(I_{3z}) = 0.0279 \pm 0.0002 \text{ s}^{-1}$, $R(2I_{1z}I_{2z}) = 0.0484 \pm 0.0004 \text{ s}^{-1}$, $R(2I_{1z}I_{3z}) = 0.0668 \pm 0.0004 \text{ s}^{-1}$, $R(2I_{2z}I_{3z}) = 0.0562 \pm 0.0004 \text{ s}^{-1}$, and $R(4I_{1z}I_{2z}I_{3z}) = 0.0689 \pm 0.0016 \text{ s}^{-1}$. The relaxation rates calculated for sucrose octaacetate from the data given in (B) are $R(I_{1z}) = 0.9701 \pm 0.0010 \text{ s}^{-1}$, $R(I_{2z}) = 0.8346 \pm 0.0007 \text{ s}^{-1}$, $R(I_{3z}) = 0.6750 \pm 0.0030 \text{ s}^{-1}$, $R(I_{4z}) = 0.7259 \pm 0.0008 \text{ s}^{-1}$, $R(2I_{1z}I_{2z}) = 1.472 \pm 0.007 \text{ s}^{-1}$, $R(2I_{2z}I_{3z}) = 1.411 \pm 0.008 \text{ s}^{-1}$, $R(2I_{3z}I_{4z}) = 1.292 \pm 0.004 \text{ s}^{-1}$, $R(2I_{4z}I_{5z}) = 1.897 \pm 0.011 \text{ s}^{-1}$, $R(4I_{1z}I_{2z}I_{3z}) = 1.978 \pm 0.007 \text{ s}^{-1}$, $R(4I_{2z}I_{3z}I_{4z}) = 1.813 \pm 0.005 \text{ s}^{-1}$, and $R(4I_{3z}I_{4z}I_{5z}) = 2.218 \pm 0.013 \text{ s}^{-1}$.

TABLE 1
Analysis of Sucrose Octaacetate Relaxation Data

Spin pair	Mutual dipolar relaxation, s^{-1} ^a	Fraction (NMR) ^b	Internuclear distance, Å (X-ray) ^c	Fraction (X-ray) ^d	Internuclear distances, Å (NMR) ^e
H1–H2	0.333 (7)	0.311 (6)	2.478	0.324	2.495 (8)
H2–H3	0.098 (8)	0.092 (8)	3.155	0.076	3.057 (42)
H3–H4	0.109 (5)	0.102 (5)	2.955	0.113	3.005 (25)
H1–H3	0.070 (12)	0.066 (11)	3.672	0.031	3.231 (90)
H2–H4	0.215 (11)	0.201 (10)	2.668	0.208	2.684 (22)
H3–H5	0.245 (18)	0.229 (17)	2.591	0.248	2.626 (33)

^a Calculated using Eqs. [12] and [13].

^b Calculated as a fraction of the sum of the mutual dipolar relaxation rates given in column 2.

^c Ref. (24).

^d The equivalent of column 3 calculated using the internuclear distances given in column 4.

^e Assuming no internal motion.

active spins are inverted to eliminate the effects of cross relaxation with the longitudinal magnetization of its active spins. These are likely to be the most important processes in this instance as the amplitudes of longitudinal magnetization will be perturbed by the preparation sequence of the experiment. The spin s for which $1/(r_{si}^3 r_{sj}^3)(3 \cos^2 \theta_{si,sj} - 1)/2$ is likely to be largest (Eq. [4]), where s , i , and j are the active spins, is inverted twice. The effects of cross relaxation with other three-spin modes in which the two inverted spins are not active are also suppressed by this procedure.

The quality of the data and hence the effectiveness of cross-relaxation suppression is assessed in Table 1. Here Eqs. [12] and [13] are used to isolate the mutual dipolar relaxation of specific pairs of spins. Each pairwise interaction is also expressed as a fraction of the sum of all of the interactions considered. This facilitates comparison with the results that would have been expected on the basis of internuclear distances obtained from the X-ray crystal structure data (24), which are also expressed in this fractional form. Internuclear distances calculated from the NMR data are also given. For all but the most widely separated pair of protons considered, the NMR data yields internuclear distances within 0.1 Å of the values expected from X-ray crystal data, and the four closest spin pairs all yield values within 0.05 Å. However, the experimental errors derived from the NMR data appear to underestimate the overall error for all but the last two spin pairs considered by a factor of at least two. It is difficult to attribute this discrepancy to one source, although unsuppressed cross-relaxation pathways and the uncompensated for nonideal behavior of some of the selective inversion pulses have probably contributed.

It was noted above that the incorporation of cross-relaxation-suppression procedures into pulses sequences for measuring the decay of longitudinal modes should make the latter less sensitive to short recovery delays ($< 5T_1$) between experiments. This is demonstrated experimentally for I_{2z} of allyl bromide in Fig. 8. The selective inversion-recovery ex-

periment incorporating cross-relaxation suppression shows deviations in the measured rate of little more than 2% for relaxation times down to a single T_1 ; at this stage, the conventional selective-inversion-recovery experiment exhibits an error of nearly 10%. At shorter relaxation times, rates calculated using the suppressed experiment show significant errors, though less than those obtained with the conventional experiment.

Data for $I_{1z} - I_{3z}$ of allyl bromide acquired with the multiple selective-inversion-recovery pulse sequence given in Fig. 2B, together with that acquired using a conventional nonselective-inversion-recovery experiment for comparison, are given in Fig. 9. The multiple selective data were acquired using the cross-relaxation suppression sequence given in Eq. [7] repeated twice, where $i = H1$ and $j = H3$. While H2 is not itself inverted by this sequence, all of the major species with which it undergoes cross relaxation are, and consequently, the effects of cross relaxation should be effectively suppressed for I_{2z} as well as for I_{1z} and I_{3z} . The conventional nonselective-inversion-recovery experiment produced rates for H1–H3 that differ from those obtained from the selective cross-relaxation-suppressed data given in Fig. 7A by +25.5, +26.1, and +16.8%, respectively. However, the rates calculated from the multiple-selective data differ from the latter by only +1.0, +1.3, and 2.5%, respectively, indicating that the cross-relaxation-suppression procedure used in the multiple-selective experiment is largely effective. If the cross-relaxation-suppression scheme given in Eq. [7] is not repeated, these errors change to +1.0, –1.6, and +6.5%. This procedure clearly has the greatest effect on the decay rate of I_{3z} because its major cross-relaxation processes are now being suppressed by a single $180^\circ(3)$ pulse. The smaller effect on the rate of I_{2z} probably reflects the reduced efficiency with which cross relaxation between I_{2z} and I_{3z} is suppressed.

CONCLUSION

We have shown that it is possible to effectively suppress the effects of cross relaxation in the auto-relaxation measure-

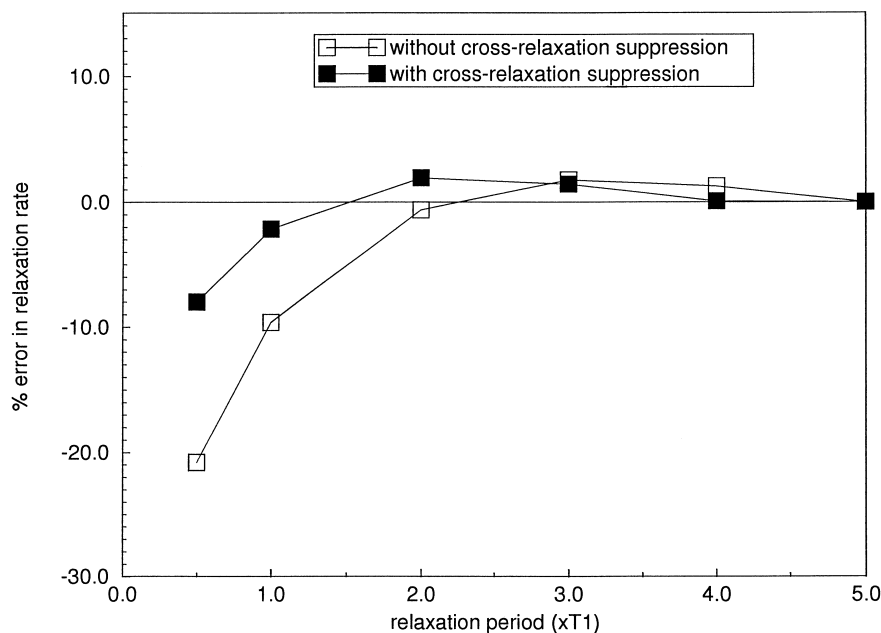


FIG. 8. Variation of the ^1H longitudinal relaxation rate with relaxation time for H2 of 0.1 M allyl bromide in CDCl_3 . Data were measured using a selective-inversion-recovery experiment with and without cross-relaxation suppression. Data were measured at 250 MHz.

ments of longitudinal modes by incorporating sequences of selective-inversion pulses into the mixing periods of the relevant pulse sequences. In theory, the major limitations of this technique are that the multiplets of the spins concerned must be resolved from each other and that the effects of cross relaxation to a species containing two equivalent spins can-

not be suppressed. In practice, imperfect inversions may also reduce the efficiency of suppression, although this can be compensated for by ensuring that each spin that is inverted is done so an even number of times. The use of cross-relaxation-suppression procedures has the effect of making experiments robust with respect to short relaxation times and can also enable a number of selective relaxation measurements to be made simultaneously in the same experiment. The availability of accurate auto-relaxation rates also makes possible their use to determine internuclear distances between sets of coupled spins; this is in contrast to conventional methods which utilize cross-relaxation rates.

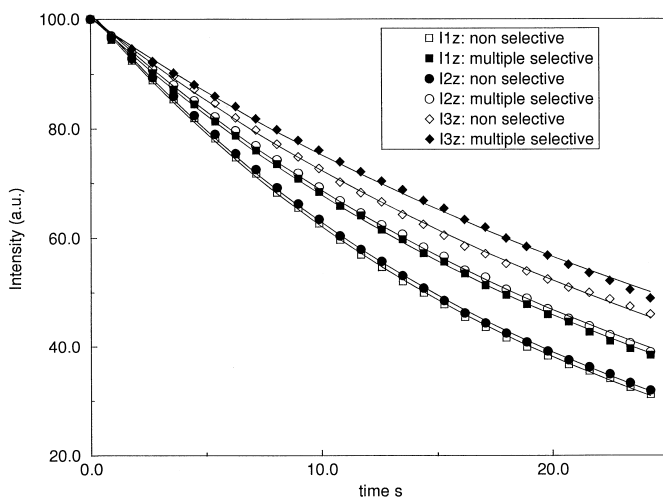


FIG. 9. The 400 MHz ^1H longitudinal relaxation data for H1–H3 of 0.1 M allyl bromide in CDCl_3 obtained with a conventional inversion-recovery pulse sequence and the multiple-selective-inversion-recovery pulse sequence given in Fig. 2B, as indicated. The relaxation rates calculated from the inversion-recovery data are $R(I_{1z}) = 0.0487 \pm 0.0002 \text{ s}^{-1}$, $R(I_{2z}) = 0.0478 \pm 0.0002 \text{ s}^{-1}$, and $R(I_{3z}) = 0.0326 \pm 0.0002 \text{ s}^{-1}$, while those calculated from the multiple-selective data are $R(I_{1z}) = 0.0392 \pm 0.0002 \text{ s}^{-1}$, $R(I_{2z}) = 0.0384 \pm 0.0002 \text{ s}^{-1}$, and $R(I_{3z}) = 0.0286 \pm 0.0002 \text{ s}^{-1}$.

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