

# Selective Detection of Biexponential Relaxation in Isotropic Solutions by Mixed Coherence Filtering NMR

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**This is a trial to simplify sequences to get the relaxation function  $f_{31}(t)$ , which is usually detected by the double- or triple-quantum filtered (DQF or TQF) NMR in an isotropic phase. The results indicate that only two phase cycling is needed to get the  $f_{31}(t)$  function, which shows the advantage of  $S/N$  (signal-to-noise ratio) in a minimum acquisition time. The filtering of coherence levels in the creation time need not be limited to double- or triple-quantum coherences. An  $^{87}\text{Rb}$  signal from a 4% (w/w) agar powder in 500-mM RbCl solution is recorded at 130.9 MHz as a Larmor frequency using a Bruker MSL 400 spectrometer. © 1998 Academic Press**

## INTRODUCTION

In many biological systems, the nature of multiexponential decay of an  $^1\text{MR}$  signal, which usually has two components (the satellite and the central transitions) in an isotropic slow motion system has been used to analyze ionic motion. The condition of an isotropic slow motion is the relation of  $\omega_Q < \tau_c^{-1} < \omega_0$  where  $\varphi_Q$  and  $\varphi_0$  are the quadrupolar splitting and Zeeman frequency and  $\tau_c$  is a correlation time between the nuclear electric quadrupole moment and fluctuating electric field gradients. The usual double- or triple-quantum filtered (DQF or TQF) NMR has been used as a powerful tool to analyze the double-exponential decay in this system. An advantage of multiple-quantum filtering NMR is the new spectra of the relaxation function  $f_{31}(t) = \sqrt{6}/5(\exp(R_1 t) - \exp(R_2 t))$ , versus the relaxation function  $f_{11}(t) = \frac{1}{5}(3\exp(R_1 t) + 2\exp(R_2 t))$  derived by the conventional single-pulse NMR. The key to analyzing the isotropic slow motion phase of the spin 3/2 system was the double-exponential treatment of FID (1, 2, 4–11, 14–16).

This paper shows a trial sequence to simplify sequences to get the relaxation function  $f_{31}(t)$  in an isotropic phase. The results indicate that only two phase cycling is enough to get the  $f_{31}(t)$ , and filtering of coherence levels in the creation time

need not be limited to double- or triple-quantum coherences. We call a sequence a mixed coherence transfer (MCT) sequence which is composed of two steps of phase cycling and its coherence levels include all levels. DQF sequences may be composed of at least two steps, however, it is usually used in 8-step phase cycling.

The  $^{87}\text{Rb}$  signals from 4% (w/w) agar powder in 500-mM RbCl solution are recorded at 130.9 MHz as a Larmor frequency using with a Bruker MSL400 spectrometer. The sample with rubidium is a typical sample of biological systems which acts biexponentially to transverse relaxation characters (9).

## THEORY

A new filtration (*mixed coherence transfer*, MCT) sequence consisting of three  $\pi/2$  pulses and one refocus  $\pi$  pulse is demonstrated here. The sequence is

$$(\pi/2)_{\varphi_1-t_1/2}-(\pi)_{\varphi_\pi-t_1/2}-(\pi/2)_{\varphi_2-(t_2)}-(\pi/2)_{\varphi_3-t_3}(\text{Acq.})_{\varphi_R},$$

where the phases  $\varphi_1$ ,  $\varphi_\pi$ ,  $\varphi_2$ ,  $\varphi_3$ , and  $\varphi_R$  are phases of each pulse and a receiver, and its phase cyclings are listed in Table 2. The creation time  $t_2$  is taken into consideration in calculating the density matrix of this system, but  $t_2$  is zero finally in the MCT experiment.

The evolution of the density matrix of this spin-3/2 isotropic system can be calculated as follows: A density operator in the thermal equilibrium state is expressed with the irreducible tensor operators  $T_{10}$  and  $T_{00}$ . The notations 1 and  $p$  in the operator  $T_{1p}$  correspond to a rank and a coherence level which in turn are related to modulations of free precession with transverse relaxation, and to RF pulse, respectively.  $T_{00}$  can be ignored anytime, because it is represented with a unit matrix which does not change by any modulation and which cannot be detected at acquisition time.  $T_{10}$ , on the other hand, transfers into  $T_{11}$  and  $T_{1-1}$  by the first  $\pi/2$  pulse. The coherence levels  $p$  and  $-p$  in the tensor operators

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**TABLE 1**  
**Calculated Results of  $\alpha$  and  $\beta$  in Eq. [4] for a Pulse Sequence Composed of Three 90° Pulses and a Refocus 180° Pulse**

$\phi_1$	$\phi_2$	$\phi_3$	$\phi_R$	$\alpha$	$\beta$	$p$	$\phi_1$	$\phi_2$	$\phi_3$	$\phi_R$	$\alpha$	$\beta$	$p$
0	0	0	0	0	0	even	0	2	0	0	0	0	even
0	0	0	1	$s$	$s$	even	0	2	0	1	$-s$	$-s$	even
0	0	0	2	0	0	even	0	2	0	2	0	0	even
0	0	0	3	$-s$	$-s$	even	0	2	0	3	$s$	$s$	even
0	0	1	0	$s/4$	$-s$	even	0	2	1	0	$-s/4$	$s$	even
0	0	1	1	0	0	even	0	2	1	1	0	0	even
0	0	1	2	$-s/4$	$s$	even	0	2	1	2	$s/4$	$-s$	even
0	0	1	3	0	0	even	0	2	1	3	0	0	even
0	0	2	0	0	0	even	0	2	2	0	0	0	even
0	0	2	1	$-s$	$-s$	even	0	2	2	1	$s$	$s$	even
0	0	2	2	0	0	even	0	2	2	2	0	0	even
0	0	2	3	$s$	$s$	even	0	2	2	3	$-s$	$-s$	even
0	0	3	0	$-s/4$	$s$	even	0	2	3	0	$s/4$	$-s$	even
0	0	3	1	0	0	even	0	2	3	1	0	0	even
0	0	3	2	$s/4$	$-s$	even	0	2	3	2	$-s/4$	$s$	even
0	0	3	3	0	0	even	0	2	3	3	0	0	even
0	1	0	0	0	0	odd	0	3	0	0	0	0	odd
0	1	0	1	0	0	odd	0	3	0	1	0	0	odd
0	1	0	2	0	0	odd	0	3	0	2	0	0	odd
0	1	0	3	0	0	odd	0	3	0	3	0	0	odd
0	1	1	0	0	0	odd	0	3	1	0	0	0	odd
0	1	1	1	$-s$	$-s$	odd	0	3	1	1	$-s$	$-s$	odd
0	1	1	2	0	0	odd	0	3	1	2	0	0	odd
0	1	1	3	$s$	$s$	odd	0	3	1	3	$s$	$s$	odd
0	1	2	0	0	0	odd	0	3	2	0	0	0	odd
0	1	2	1	0	0	odd	0	3	2	1	0	0	odd
0	1	2	2	0	0	odd	0	3	2	2	0	0	odd
0	1	2	3	0	0	odd	0	3	2	3	0	0	odd
0	1	3	0	0	0	odd	0	3	3	0	0	0	odd
0	1	3	1	$-s$	$-s$	odd	0	3	3	1	$-s$	$-s$	odd
0	1	3	2	0	0	odd	0	3	3	2	0	0	odd
0	1	3	3	$s$	$s$	odd	0	3	3	3	$s$	$s$	odd

*Note.*  $\phi_1, \phi_2, \phi_3,$  and  $\phi_R$  are phases of the three 90° pulses and receiver phases. Phases of the 180° pulses are set to be the same as that of the first 90° pulse ( $\phi_1$ ).  $s$  stands for  $\sqrt{5}$  in this table.  $p$  represents the coherence level just after the second 90° pulse, *even* represents 0,  $\pm 2$ , and *odd* represents  $\pm 1, \pm 3$ .

appear in pairs, so that we will discuss the positive terms for simplifying discussions of evolutions of the density operator of this system. The negative terms will be taken into account in the final results.

During the evolution time  $t_1$  the operator  $T_{11}$  is converted into  $T_{31}$  and  $T_{11}$  dressed with relaxation functions  $f_{31}(t_1)$  and  $f_{11}(t_1)$  as coefficients, respectively. Note that a second rank and single-quantum tensor  $T_{21}$  cannot be created during any precession period with relaxation in the isotropic system (2, 13, 17). Explicit forms of the functions  $f_{31}(t)$  and  $f_{11}(t)$  are expressed as

$$f_{31}(t) = f_{13}(t) = \frac{\sqrt{6}}{5} (e^{R_1 t} - e^{R_2 t}) \quad [1]$$

$$f_{11}(t) = \frac{1}{5} (3e^{R_1 t} + 2e^{R_2 t}), \quad [2]$$

where  $R_1$  is the satellite transition rate and  $R_2$  is the central one (2). The refocus  $\pi$  pulse has no effect on this density matrix except that of reversing the coherence levels.

The second  $\pi/2$  pulse produces another set of tensor operators that represent various kinds of coherence orders, i.e.,  $T_{11}$  is converted into  $T_{11}$  or  $T_{10}$  and  $T_{31}$  into  $T_{33}, T_{32}, T_{31}$  or  $T_{30}$ . The relaxation during the creation time  $t_2$ , along with the third  $\pi/2$  pulse, serves to modulate the density operator and to create various other tensor operators dressed with proper relaxation functions. If we simply take into account all tensors having all ranks through this pulse sequence, the final detectable density operator during an acquisition time  $t_3$  is expressed with the nine terms

$$\begin{aligned} \sigma(\varphi_1, \varphi_2, \varphi_3, t_1, t_2, t_3) = & Af_{11}(t_1)f_{11}(t_2)f_{11}(t_3)T_{11} \\ & + Bf_{11}(t_1)f_{21}(t_2)f_{12}(t_3)T_{11} \\ & + Cf_{11}(t_1)f_{31}(t_2)f_{13}(t_3)T_{11} \end{aligned}$$

$$\begin{aligned}
& + Df_{21}(t_1)f_{12}(t_2)f_{11}(t_3)T_{11} \\
& + Ef_{21}(t_1)f_{22}(t_2)f_{12}(t_3)T_{11} \\
& + Ff_{21}(t_1)f_{32}(t_2)f_{13}(t_3)T_{11} \\
& + Gf_{31}(t_1)f_{13}(t_2)f_{11}(t_3)T_{11} \\
& + Hf_{31}(t_1)f_{23}(t_2)f_{12}(t_3)T_{11} \\
& + If_{31}(t_1)f_{33}(t_2)f_{13}(t_3)T_{11}, \quad [3]
\end{aligned}$$

where  $A, B, C, \dots, I$  are determined by phase sets of  $(\varphi_1, \varphi_2, \varphi_3)$ . It would appear that several of the terms in Eq. [3] can be overlooked for the following reasons. The terms of  $D, E$ , and  $F$  should not be created in this isotropic system because  $T_{21}$  does not exist during evolution time  $t_1$ , as mentioned above. For the same reason—that is, the absence of  $T_{21}$  during the acquisition period  $t_3$ —the terms  $B$  and  $H$  can also be ignored. It should be noted here that if we set the creation time  $t_2$  to be zero, then  $f_{31}(t_2)$  and  $f_{13}(t_2)$  will be zero, and  $f_{33}(t_2)$  and  $f_{11}(t_2)$  will be 1 (the explicit form of  $f_{33}(t)$  is  $f_{33}(t) = \frac{1}{5}(2 \exp(R_1 t) + 3 \exp(R_2 t))$ ). Thus the final density operator includes only the two terms  $A$  and  $I$ .

We can now derive the final signal at acquisition time  $t_3$  as a trace of a product of the  $\sigma$  and shift operators  $I^\pm = I_x \pm iI_y$ . The final result is

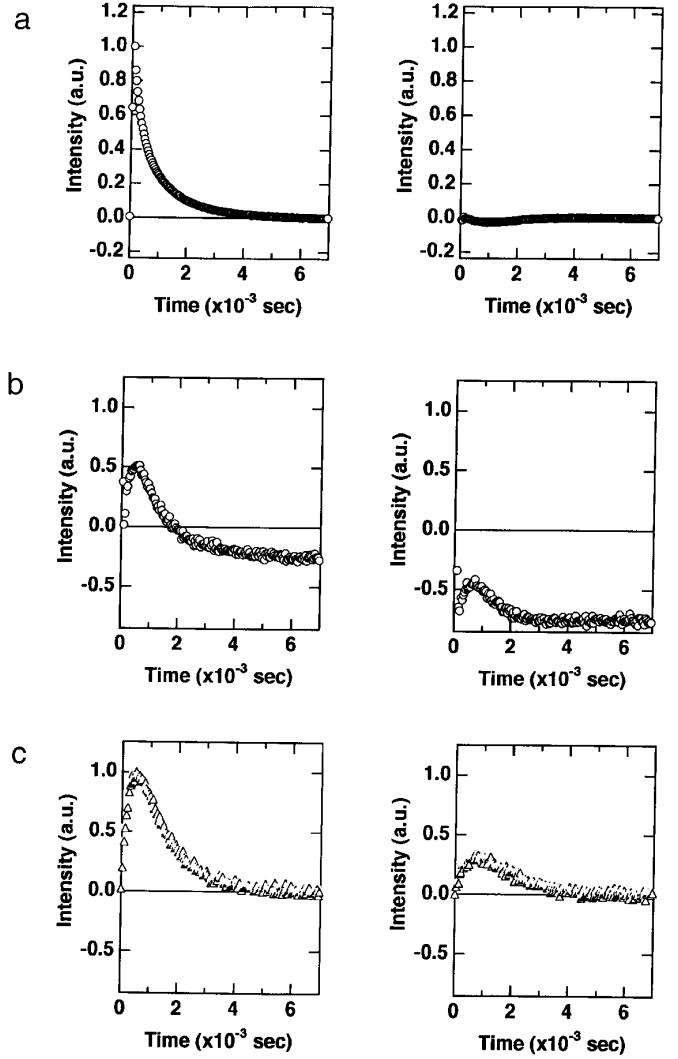
$$\begin{aligned}
& \text{Signal}(\varphi_1, \varphi_2, \varphi_3, \varphi_R, t_1, t_2, t_3) \\
& = \alpha(\varphi_1, \varphi_2, \varphi_3, \varphi_R) f_{31}(t_1) f_{13}(t_3) \\
& + \beta(\varphi_1, \varphi_2, \varphi_3, \varphi_R) f_{11}(t_1) f_{11}(t_3), \quad [4]
\end{aligned}$$

where  $\alpha$  and  $\beta$  are as listed in Table 1. The phases of the RF pulses in Table 1 are varied at  $90^\circ$  steps. The receiver phase is the same as the first  $90^\circ$  pulse ( $\varphi_1$ ).

**TABLE 2**  
Examples of Phase Cycling of the MCT Sequence

(a)								
$\varphi_1$		0						1
$\varphi_\pi$		0						1
$\varphi_2$		0						0
$\varphi_3$		1						0
$\varphi_R$		0						0
(b)								
$\varphi_1$	0	1	2	3	0	1	2	3
$\varphi_\pi$	0	1	2	3	0	1	2	3
$\varphi_2$	0	1	2	3	3	0	1	2
$\varphi_3$	1	2	3	0	3	0	1	2
$\varphi_R$	0	1	2	3	3	0	1	2

Note. (a) Examples of the simplest MCT; (b) examples including a CYCLOPS procedure.



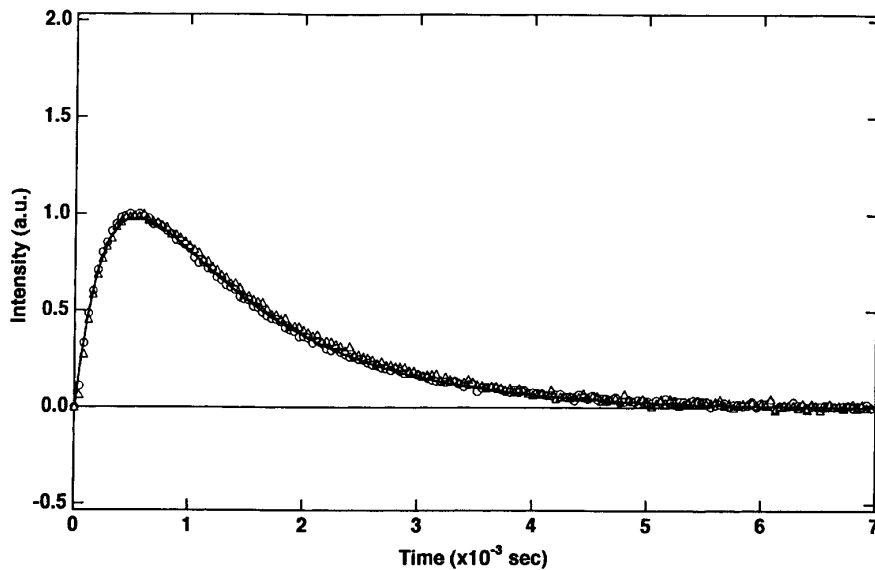
**FIG. 1.** Free induction decays of  $^{87}\text{Rb}$  in 4% (w/w) agar solution detected from sequences of (a) single pulse, (b) DQF, and (c) MCT. Number of accumulations for the single pulse experiment, as well as for the DQF and MCT experiments, was 1024. Signals of (b) and (c) are normalized by the peak intensity of the on-resonance part of FID(c). The sampling time and filtering width for all experiments were  $20 \mu\text{s}$  and  $100 \text{ kHz}$ .

Each of the signals in Table 1 can be classified according to the phase sets  $(\varphi_1, \varphi_2, \varphi_3, \varphi_R)$  as one of three types:

$$\text{type 1: Signal}_1 = 0 \quad [5]$$

$$\begin{aligned}
& \text{type 2: Signal}_2 = \alpha_2 f_{31}(t_1) f_{13}(t_3) + \beta_2 f_{11}(t_1) f_{11}(t_3) \\
& = \mp \frac{\sqrt{5}}{4} f_{31}(t_1) f_{13}(t_3) \pm \sqrt{5} f_{11}(t_1) f_{11}(t_3) \quad [6]
\end{aligned}$$

$$\begin{aligned}
& \text{type 3: Signal}_3 = \alpha_3 f_{31}(t_1) f_{13}(t_3) + \beta_3 f_{11}(t_1) f_{11}(t_3) \\
& = \pm \sqrt{5} f_{31}(t_1) f_{13}(t_3) \pm \sqrt{5} f_{11}(t_1) f_{11}(t_3). \quad [7]
\end{aligned}$$



**FIG. 2.** Comparison of free induction decays using MCT (open circles) and DQF (triangles) sequences for  $^{87}\text{Rb}$  in 4% (w/w) agar solution. A solid line is fitted to the free induction decay of the MCT sequence with a function of  $f_{31}$ , where  $R_1 = -3235 \text{ s}^{-1}$  and  $R_2 = -846 \text{ s}^{-1}$ . Both signals are scaled to the peak intensity of FID from the MCT experiment.

Subtracting  $\text{Signal}_3$  from  $\text{Signal}_2$  makes a pure  $f_{13}(t_3)$  function,

$$\frac{\text{Signal}_2 = \alpha_2 f_{31}(t_1) f_{13}(t_3) + \beta_2 f_{11}(t_1) f_{11}(t_3)}{(-)\text{Signal}_3 = \alpha_3 f_{31}(t_1) f_{13}(t_3) + \beta_3 f_{11}(t_1) f_{11}(t_3)}, \quad [8]$$

$$\text{Signal}(t_1, t_3) = \alpha' f_{31}(t_1) f_{13}(t_3)$$

where  $\alpha' = \alpha_2 - \alpha_3$  and  $\beta_2 = \beta_3$ .

It should be emphasized that two phase cyclings are enough to derive the relaxation function  $f_{31}(t)$ . The coherence order just after the second  $90^\circ$  pulse of  $\text{Signal}_2$  and parts of  $\text{Signal}_3$  is always an *even* integer, such as 0 or  $\pm 2$ , and therefore may lead to a DQF sequence, although the usual DQF sequences are composed of 8 steps in phase cycling (18).

MCT is composed of *even* coherence types  $\text{Signal}_2$  and *odd* coherence types  $\text{Signal}_3$  (see Table 2). It includes all coherences 0,  $\pm 1$ ,  $\pm 2$ ,  $\pm 3$  during the creation time. This is the first sequence to get the  $f_{31}(t)$  using mixed coherence pathways. If these kind of 2-step sequences are used in a minimized accumulation experiment, clearly the signal-to-noise ratio will be four times better than that by the usual 8-step DQF sequence.

We assumed that this system was purely isotropic. As mentioned below (under Experimental), anisotropic phase signals can produce artifacts during the 2-step phase cycling sequence to derive  $f_{31}(t)$ . Fortunately, however, we know that artifacts of the anisotropic phase signals, such as remaining quadrupolar splittings, will appear only in the offset phase of FID (13, 17). By carefully adjusting the carrier frequency to the center of a spectrum from a single pulse experiment, we can prevent such artifacts from affecting the main phase of FID.

## EXPERIMENTAL PROCEDURE AND RESULTS

Agar powder (4.0% w/w) was dissolved in 500-mM RbCl solution prior to the experiments (9). The  $^{87}\text{Rb}$  signals were recorded at 130.9 MHz as Larmor frequencies using a Bruker MSL400 spectrometer at room temperature. The  $\pi/2$  pulse used (15.0  $\mu\text{s}$ ) was short and the filter width (100 kHz) wide enough to guarantee uniform excitation of the entire  $^{87}\text{Rb}$  resonance.

Figure 1 shows free induction decays from a single pulse, and from the DQF and MCT sequences, which clearly show the characteristic of double exponential decays. The 2-step phase cycling experiment such as MCT is more sensitive to phase offset or may be also more sensitive for information from anisotropic phase. There may be other possibilities to know different types of information using the 2-step phase cycling procedure. A receiver phase and the carrier frequency were adjusted by carefully referencing a peak of a spectrum of a single pulse experiment. If a CYCLOPS sequence is used with the MCT experiment, the additional sensitivity such as phase offset and RF inhomogeneity will disappear. The left-hand side of Fig. 1 shows the on-resonance part of FID, and the right-hand side shows the offset side. The number of accumulations for the single pulse experiments was 1024. Both the DQF and MCT experiments were accumulated 1024 times. The experiments in Figs. 1 and 2 indicate that the sensitivities of DQF and MCT (see on-resonance side of FID) are about the same. The FID of the MCT sequence in Fig. 1c includes DC offset on its baseline, while the FID of the DQF experiment in Fig. 1b does not. The MCT sequence used in Fig. 1c is the simplest sequence (Table 2a) not containing the CY-

CLOPS procedure. The MCT experiment in Fig. 2 was recorded by the sequence in Table 2b, which includes a CYCLOPS procedure. The CYCLOPS procedure may not be needed if the spectroscopic facilities are precisely tuned. It seems that the use of the MCT sequence has no advantages over that of the DQF sequence, particularly in experiments requiring many accumulations. It is worth noting that the MCT sequence derives the function  $f_{31}(t)$  most simply. Two phase cyclings are sufficient to provide the relaxation function  $f_{31}$ , while the usual DQF requires eight phase cyclings. If an experiment has been done with no accumulation, MCT will be at least 4 times more sensitive than the usual simplest accumulation used in the DQF sequence, though the presence of numerous accumulations will reduce this advantage. If, in the future, the sensitivity of the spectroscopic environment were itself enhanced, then MCT would have some advantages over DQF. The 20- $\mu$ s sampling time used in this experiment and 100-kHz filtering width are the same for each experiment.

We compare the FID of MCT (open circles) and DQF (triangles) in Fig. 2. A solid line is fitted to the free induction decay of the on-resonance part of the MCT sequence with a function of  $f_{31}$ , where  $R_1 = -3235 \text{ s}^{-1}$  and  $R_2 = -846 \text{ s}^{-1}$ . The MCT experiment used the sequence in Table 2b. The number of accumulations in these experiment was 10,240. The other experimental parameters were the same as those of Fig. 1. Our MCT sequence appeared to filter the function  $f_{31}$ , and the information from the isotropic part (onresonance) appeared to be completely separated from the anisotropic (off-resonance) part.

Table 1 shows the ideal signals from the sequence mentioned above. The phase of refocus  $180^\circ$  pulse is the same as the first  $90^\circ$  pulse and reflects any influence on the theoretical calculations.  $s$  stands for  $\sqrt{5}$  in this table. The  $p$  are coherence levels just after the second  $90^\circ$  pulse, *even* represents 0,  $\pm 2$ , and *odd* represents  $\pm 1$ ,  $\pm 3$ . One can easily produce a variety of MCT sequences from this table. Table 2 is an example of an MCT sequence used in this experiment.

## CONCLUSION

A new selective detection method that can filter a relaxation function  $f_{31}(t)$  of a spin  $3/2$  system in isotropic phase has been demonstrated. The MCT sequence is composed of three  $90^\circ$  pulses and a refocus  $180^\circ$  pulse, and it requires at least two phase cyclings to yield the relaxation function  $f_{31}(t)$ , while

DQF typically requires eight phase cyclings. When a substantial number of accumulation is required, the signal-to-noise ratio of MCT seems to be equal to that of DQF. It is possible that, in the future, MCT will be four times as sensitive as DQF. The MCT with no CYCLOPS sequence is more sensitive to the phase offset or the information from anisotropic phase, which is separated in the off-resonance part. The NMR signals of  $^{87}\text{Rb}$  in 4% (w/w) agar solution were recorded as a typical sample of an isotropic and slow motion phase. Our results also showed that filtering of coherence levels in the creation time need not be limited to double- or triple-quantum coherences.

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