

Comparative Studies of Phase-Cycling Schemes for Multiple π -Pulse Sequences

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Recently, a new phase cycling scheme was introduced by this laboratory for use in biological solid-state NMR experiments involving multiple π -pulses with characteristics that suggested it may enhance the sensitivity of these kind of experiments (Y. Li and J. N. S. Evans, 1995, *Chem. Phys. Lett.* 241, 79 and Erratum, 1995, *ibid.* 246, 527; Y. Li and J. N. S. Evans, 1996, *J. Magn. Reson. B* 111, 296). The new sequence followed the supercycled concept proposed a decade ago for heteronuclear decoupling experiments. In this paper, more detailed experiments demonstrate that the claim of enhanced sensitivity was unfounded, and in fact the supercycle proposed differs little from the established XY-8 and XY-16 based supercycles. © 1997 Academic Press

Many NMR experiments, including the methods for internuclear distance measurements (1, 2), make use of equally spaced π -pulses. To compensate for imperfections in the π -pulses, several phase-cycling schemes have been proposed (3–5). It was shown by Waugh (6), using the decoupling sequences as an example, that by concatenating the basic cyclic unit with either the cyclically permuted sequence or the phase-inverted sequence, errors in the concatenated sequence are reduced when compared to the simple repeat of the basic cyclic unit. The XY phase-cycling schemes, proposed by Gullion *et al.* (5) are constructed according to the above rule. They were shown to successfully eliminate the off-resonance effects in the REDOR experiment (7) and refocus all three components of the magnetization (5). Recently, a new phase-cycling scheme has been introduced by this laboratory (8, 9). It relies on the same supercycled concept as the XY schemes do. This phase-cycling scheme (referred to in this article as the supercycle) was suggested to be superior to XY phase-cycling schemes, although no accurate comparative study has been performed. In this work, we attempted to compare the performance of the two types of schemes and identify the advantages they may offer when used in appropriate NMR experiments.

The sample used for the experiments is sodium ammonium phosphate. Solid-state NMR was carried out on a Chemagnetics CMX-400 instrument equipped with a Chemagnetics triple resonance probe. The Carr–Purcell type pulse sequence used in the experiment is shown in Fig. 1. It consists of a conventional cross-polarization followed by the train of phase-cycled π -pulses and the refocusing π -pulse. The π -pulses are synchronized with the rotor period. The π -pulse width for ³¹P was 8.3 and 16.6 μ s, and for ¹H was 6.4 μ s. The cross-polarization field strengths were 60.2 and 30.1 kHz. The contact time was 1 ms in all cases. The ¹H decoupling field strength was set to 80.6 kHz during all the experiments. The recycle delay was 30 s. The spinning rate of the sample was electronically controlled with an accuracy of ± 6 Hz. In all experiments, the π -pulses were phase cycled by the supercycled and XY methods. Two sets of data corresponding to the two different phase-cycling schemes were acquired simultaneously to ensure that any instabilities in the instrument operation would affect both data sets in a similar fashion.

The simulations were obtained numerically using home-written Fortran code based on density operator formalism run on a 16-processor IBM SP2 computer. Free precession of the spin system was explicitly accounted for by use of the appropriate internal Hamiltonian. Pulses, including pulse widths, were explicitly accounted for by use of the appropriate radiofrequency Hamiltonian, as well as the internal Hamiltonian of the spin system. The CSA eigenvalues for Na (NH₄)HPO₄·2H₂O used as empirical parameters in the simulations were $R_{zz} = -57$ ppm, $R_{xx} = 37$ ppm, and $R_{yy} = 4$ ppm (10). All simulations were obtained using an 8.3- μ s pulse width. The RF field strengths used was 66.667 kHz, corresponding to a flip angle of 199°. The latter value, while high compared with experiment, was required for reasonable agreement between simulation and experiment. As noted below, not taken into account in the simulations were B_1 RF field inhomogeneity or homonuclear dipole–dipole interactions.

The phases for the schemes tested, as well as the logic of their construction, are listed in Table 1. One can easily see that high-order expansions of XY phase-cycling schemes are

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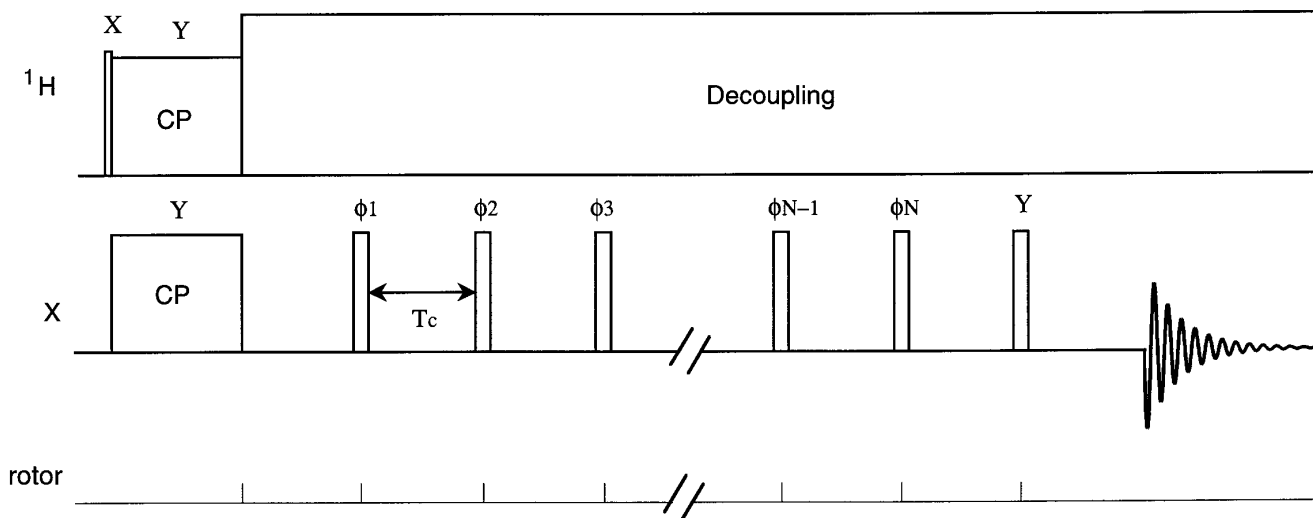


FIG. 1. Pulse sequence for the supercycled experiment. The phases of the pulses are changed according to the parameters in Table 1.

constructed by either inversion or cyclic permutation of the preceding cycle with the subsequent concatenation of the two sequences. Thus, for example, XY-128 is obtained by concatenating the basic and cyclically permuted XY-64. The supercycle is also related to the XY phase cycling schemes in the sense that it consists of the basic and phase-inverted XY-64. The evaluation of the relative performance of the cycles can be made by determining the ratio of ^{31}P isotropic peak areas, which is proportional to the magnitude of the transverse magnetization. XY-16 was chosen to be compared with the supercycle to verify the data obtained previously (8). Comparison of the XY-128 with the supercycle also appeared to be important, because both schemes contain the same number of π -pulses.

In Fig. 2A, the natural logarithm of the ratio of the ^{31}P isotropic peak areas, obtained using the supercycle, S_{SC} , to those of XY-16, S_{16} , is plotted against the number of phase-cycled π -pulses. The data shown with open circles and triangles correspond to 8.3 and 16.6 μs pulse widths respectively. The fitted line results from the simulation. The performance of XY-16 becomes worse as the number of π -pulses and the pulse duration increase. The obvious reason for this is that the simple repeat of XY-16 causes the accumulation of error in a constructive manner, whereas in the supercycle the errors are compensated by alternating inversions and cyclic permutations. The trend of the fit of the simulation to the experimental data is quite good, although there remain slight differences between the simulated and experimental data.

In Fig. 2B, a similar plot for the supercycle and XY-128 is shown. The oscillatory behavior of $\ln(S_{\text{SC}}/S_{128})$ and the increase of the oscillation amplitude upon the increase of the π -pulse duration should be noted. This behavior can be easily explained by examining the structure of the phase-

cycling schemes. As can be seen from the Table 1, the first half of each cycle represents XY-64, while the second half is different: phase-inverted XY-64 for the supercycle and cyclically permuted XY-64 for the XY-128. Therefore, when the number of phase-cycled π -pulses exceeds 64, the cyclic properties of the two schemes become different. In the case of XY-128, the basic cyclic unit becomes equal to 64 pulses, whereas in the supercycle it remains equal to 16. Thus, XY-128 does not have to be cyclic between 64th and 128th, 192nd and 256th π -pulses, but must refocus the magnetization when the number of π -pulses is a multiple of 64. The simulated line shows these oscillatory properties, although the fit deviates from the experimental data in places. Possible causes are: (i) B_1 RF field inhomogeneity; or (ii) ^{31}P - ^{31}P dipole-dipole interactions. The B_1 RF field inhomogeneity was measured as 80% (resonance intensity obtained with an 810° pulse/ 90° pulse). The dipole-dipole interaction can be expected to be nonnegligible for this sample, although its magnitude was not determined for use in the simulations.

The supercycle, being a modification of XY-64, naturally performs better than XY-16 as the number of phase-cycled

TABLE 1
Logic of XY Phase Cycling Schemes
and Supercycle Construction

Define	$R = XYXY YXYX \overline{XYXY} \overline{YXYX} = XY-16$
	$R' = \text{permuted } R = \overline{XXYX} \overline{YYXY} \overline{XXYX} \overline{YYXY}$
	$Q = RR' = XY-32$
	$XY-64 = Q\overline{Q}$
	$XY-128 = Q\overline{Q} \{Q\overline{Q}\}'$
	Supercycle (SC) = $Q\overline{Q} \{\overline{Q\overline{Q}}\}$

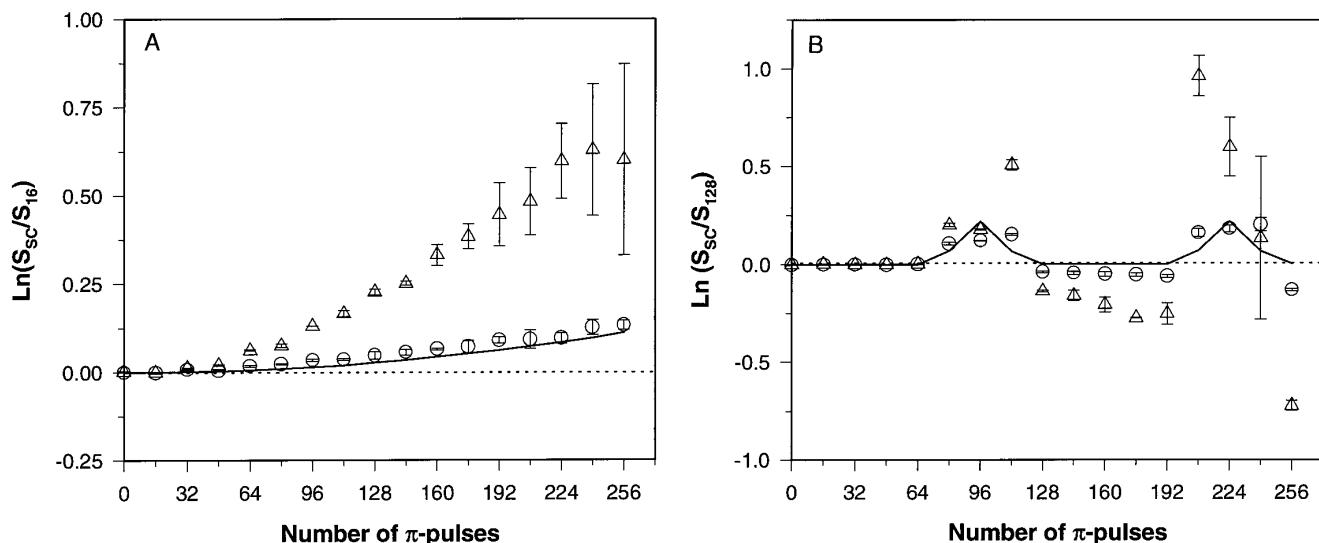


FIG. 2. Logarithm of the ratio of the ^{31}P isotropic peak areas for the two different phase-cycling schemes: (A) supercycle and XY-16, and (B) supercycle and XY-128 as a function of the number of phase-cycled π -pulses. Open circles correspond to $8.3\ \mu\text{s}$ pulse duration, open triangles to $16.6\ \mu\text{s}$.

π -pulses increases. It is worth noting that very rarely does the number of π -pulses used in most NMR experiments exceed 128. The XY-64 phase-cycling scheme, if repeated twice, should not result in a significant accumulation of error. Thus, the supercycle offers no advantage over XY-64. One might argue that the supercycle has an advantage over XY-128 when the latter is not cyclic (e.g., when the number of π -pulses is equal to 80, 96, 112—see Fig. 2B). In this case the above argument also holds—the repeat of XY-64 would probably compensate for the pulse imperfections no worse than the supercycle does. In summary, the supercycle offers no advantage over XY phase-cycling schemes and, in fact, represents the high-order expansion of these schemes with the only difference that the rule of alternating inversion and cyclic permutation is violated in the last stage of its construction. In general, there is no practical need for constructing such lengthy pulse sequences, and in most experiments XY-4, -8, or -16 perform very well and are widely used.

ACKNOWLEDGMENT

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