Coherence Selection and Excitation Sculpting Using RF-Gradients in Selective 1D Experiments and Nonselective 2D Experiments

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The B_0 gradient pulses can be replaced with long high-power pulses (RF-gradients) in z-rotation composite sandwiches. By doing this, practically all B_0 gradient-based pulse sequences can be performed with instruments lacking a field gradient accessory. We have implemented RF-gradients into selective 1D and nonselective 2D TOCSY and NOESY experiments. The spectral quality obtained with the RF-gradient method was comparable to that of the B_0 method for small- to medium-sized molecules. There are also some advantages in using RF-gradients instead of B_0 gradients. There is practically no shift or coupling evolution during RF-gradient pulses. This may be significant in some experiments. © 1998 Academic Press

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INTRODUCTION

Selective 1D versions of TOCSY (*1–4*) and NOESY (*1, 2, 5–7*) offer a short measurement time and good resolution. The information obtained from these experiments often solves the usual assignment verification problems encountered in the ''spectroscopic phase'' of synthetic work. Most of the finest methods require gradient capabilities (*3–7*) and some necessitate the calibration of the phase difference between hard and soft pulse power levels. In this paper we present radio frequency gradient (RF-gradient) (*8–12*) based methods for 1D TOCSY and NOESY which are applicable to instruments lacking the B_0-gradient unit. Further, these sequences are based on selective 180° pulses and thus avoid the problems in phase shifts. Due to their simple setup these sequences can easily be incorporated into basic set of experiments which are routinely performed by the casual NMR user. The application of the RF-gradient method to 2D-experiments (in a similar way to B_0-gradients) is also described. Previously, the RF-gradients have been applied to COSY (*9, 10*) and NOESY (*11*). Recently, we have applied them for excitation sculpting in 2D HSQC (double-SLBIRD-HSQC) (*12*).

Gradient pulses (both B_0 and RF) are used to defocus or refocus magnetization. Thus, it is possible to select desired spectral features using gradients, i.e., to destroy the unwanted

magnetization or to encode the desired coherences with spatially dependent phase during the pulse sequence and to decode them before acquisition. In theory, the dephasing effect of the gradient pulses is reversible but in practice some amount of magnetization will be lost due to diffusion effects (*13*).

There are different ways to use RF-gradients. One is to disperse magnetization in xy-plane, just like B_0-gradients along z-axis do (*8, 9*). Other possibilities include dephasing the magnetization that is not along the B_1 field axis (*14*), and using RF-gradient pulses as excitation pulses (*10, 11, 15*) and quadrupolar RF-gradients (*16*), just to mention some.

In our approach B_0 gradients along the z-axis are replaced by conventional long proton pulses of rotation angle β implemented into simple z-composite rotation clusters: $90°_{(y)} - \beta_{(-x)} - 90°_{(-y)}$ (*9, 16, 17*). More complicated clusters have been presented previously (*9*). The B_1 inhomogeneity of normal ^{1}H coil during the long β-pulse disperses the magnetization vectors in the yz-plane. This fan of vectors is then brought back into the xy-plane by the last 90° pulse of the z-composite rotation cluster. Typical lengths for RF-gradient pulses are 1–3 ms. These clusters mimic the effect of the B_0 gradients on proton magnetization and can therefore replace conventional gradient pulses in pulse sequences.

Although RF-gradients have the same effects as the B_0-gradients, there are some basic differences. First, RF-gradients are frequency selective and act on nutation rather than on precession. Dephasing of magnetization due to B_1 inhomogeneity is not a function of one particular axis as is the case with normal B_0 gradients (usually along the z-axis) (*13*). The chemical shift and coupling evolution is active during the B_0 gradient. The situation is different with RF-gradients. During the long β-pulse of the z-composite rotation cluster the magnetization is spin-locked along the B_1 field and thus practically no chemical shift or J-coupling evolution takes place. As the B_1 gradient method is based on RF-pulses, off-resonance effects cannot be avoided. Off-resonance effects will cause some loss of coherences for off-resonance spins. However, this loss is not significant when the B_1 field is strong and the chemical shift range is relatively narrow (this is usually true for proton experiments). When strong spin-lock pulses are applied the

Hartmann–Hahn condition is fulfilled. This will also lead to coherence loss, but in practice as the J_{HH}'s are around 2–20 Hz and the lengths of RF-gradient pulses on protons are on the order of 1–3 ms, the Hartmann–Hahn transfer will not cause a significant contribution. The effects of ROESY transfer are not significant either. This is, again, due to the relatively short spin-locking periods.

Diffusion can also cause a decrease in signal intensity in all gradient-based experiments. These effects are common especially with small molecules in nonviscous solvents. The effect is greatest when the gradient is used to label (dephase) the selected coherence and the rephasing is done just before acquisition (i.e., the delay between the labeling and rephasing is relatively long). The effect is particularly significant in NOE experiments with small molecules, as there is plenty of time for diffusion during the mixing time.

RESULTS AND DISCUSSION

The selective 1D TOCSY is especially valuable when a molecule contains several spin systems in which the protons can exchange magnetization during TOCSY mixing time. With long enough mixing time the whole subspectrum of the particular subspin system to which the selectively excited proton belongs can be achieved. This can be of great importance in accomplishing the assignment, especially when overlap takes place between the resonances of the separate spin systems. Further, the extracted signals (in favorable cases) can be used like a normal ^{1}H spectrum to analyze the coupling constants. The simplest way to perform selective 1D TOCSY (1, 2) is a selective 90° pulse followed by spin-lock period (for example a MLEV-17 cycle) (18). In practice, however, this method suffers from the phase difference between the soft pulse and hard pulse power levels. Phase distortions will also arise due to evolution of couplings and chemical shifts during the soft 90° pulse. A better way to selectively excite a particular resonance is to use a $(90^\circ_{(hard)} - \text{gradient} - 180^\circ_{(soft)} - \text{gradient})$ cluster. In this case, the gradient pulse can be used either to dephase all magnetization except that of the desired one (excitation sculpting) (19) or to encode the desired magnetization and decode it before acquisition (gradient selection). The basic advantage of this approach is that the calibration of the 180° soft pulse length is easier than the length of 90° soft pulses and there is no need for calibration of the phase relation to hard pulses. The selective 1D TOCSY with gradient-echo cluster can also be performed using RF-gradients, produced by normal ^{1}H coil instead of conventional B_0 gradients. For 1D TOCSY both excitation sculpting and selection with RF-gradients are applicable.

Excitation Sculpting Method for Selective 1D TOCSY

In the excitation sculpting method (19, 20), the unwanted magnetization is destroyed by the RF-gradients placed on both sides of selective 180° pulse and the desired magnetization is not affected. This method preserves the coherence order. After the sculpting (one or two aforementioned clusters) step, the conventional MLEV-17 with trim pulses on both sides is applied for appropriate time. The pulse sequence for selective 1D RFG-TOCSY (1D radiofrequency gradient TOCSY) is shown in Fig. 1A. To reinforce the echo formation, the first selective pulse was phase cycled using EXORCYCLE (21). The selective 1D RFG-TOCSY spectra (Fig. 2) were recorded from 0.5 M sucrose in D_2O at 298 K by selectively exciting the anomeric proton and using the same receiver gain in all experiments. Four-step EXORCYCLE is not necessarily needed, as the first two steps of EXORCYCLE give an acceptable spectrum (Fig. 2). There is practically no difference between the spectra obtained using RF- or B_0 gradients when one of the selective pulses is phase cycled. If a single-scan spectrum is needed, the B_0 gradient version performs better, but the suppression of the unwanted signals is not complete. As two scans become acceptable, the performances of these two experiments becomes practically equal. The differences in linewidths between RF- and B_0 gradient methods (lines are narrower in RF-gradient experiments!) are possibly due to the fact that with the RF-gradient method the lock system is enabled throughout the experiment, whereas in case of the B_0 method, the lock system is enabled only during the relaxation delay, resulting in broader lines.

Figure 3 presents selective 1D TOCSY spectra of sucrose recorded with the double-echo sequence shown in Fig. 1A and the corresponding B_0-gradient version using different mixing times. Figure 4 shows the quality of single echo method (Fig. 1A, second echo omitted). As can be seen in Fig. 4, the optimization of the spin-lock pulse length to achieve a continuous distribution over all effective rotation angles (to enforce RF-inhomogenity) can yield a significant improvement in signal intensity.

Coherence Selection Using RF-Gradients for Selective 1D TOCSY

RF-gradients can also be used for coherence selection in selective 1D RFG-TOCSY experiment (sequence in Fig. 1B). In this case the RF-gradients have opposite polarity (i.e., the long pulses on both sides of the selective pulse have opposite phases). This cluster labels the selectively inverted magnetization with twofold phase encoding. The subsequent MLEV-17 with trim pulses on both sides transfers the labeled magnetization through the spin system. The phase decoding is performed by implying the RF-gradient of double length after the mixing sequence. This method also works fine and basically only a two-step phase cycle is needed, as was also the case for excitation sculpting method. Now, however, due to the *selection* method, half of the signal will be lost, as only one of the two coherence pathways is selected. The selective 1D RFG-TOCSY spectrum of sucrose recorded using the coherence selection method (sequence in Fig. 1B) is presented in Fig. 5.
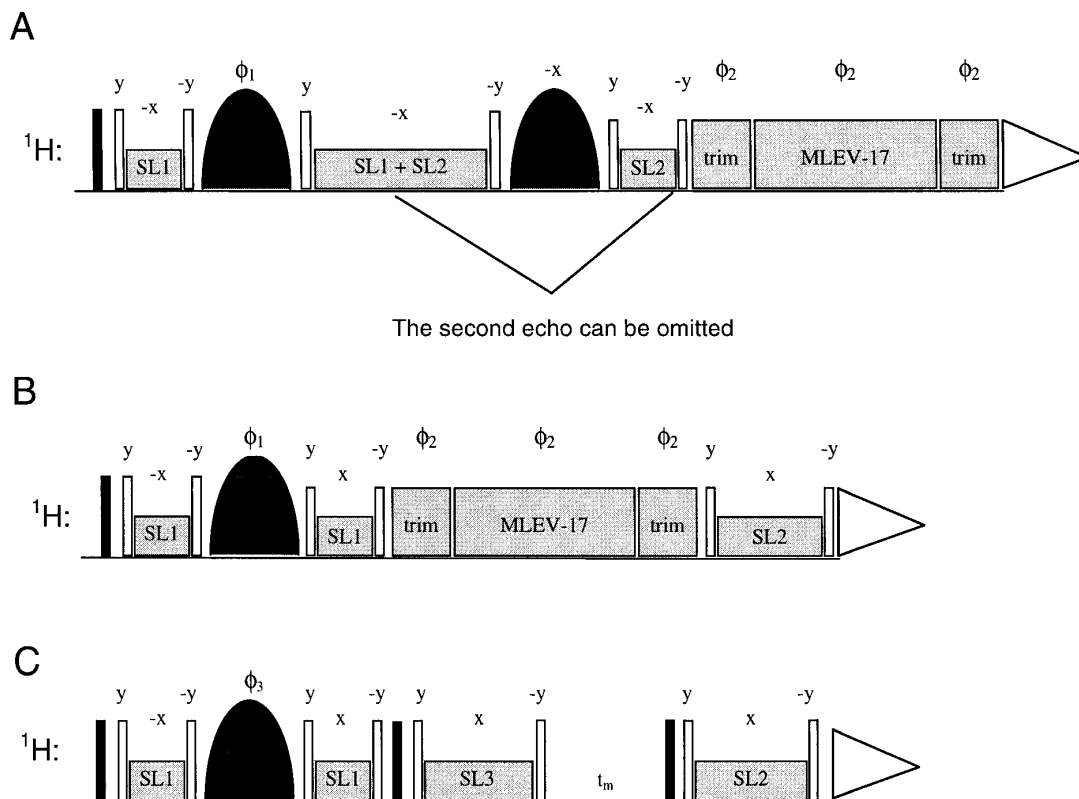
A

The second echo can be omitted

B

C

FIG. 1. Pulse sequences for the selective 1D RFG-TOCSY, excitation sculpting approach (A), the RF-gradient selection approach (B), and the selective 1D RFG-NOESY approach (C). Narrow black and white bars indicate 90° hard rectangular pulses in the basic sequence and in z-rotation clusters, respectively. The long β-pulses are represented by wide gray bars denoted ''SL.'' Selective 180° pulses are represented by dark gray half-ellipses. Wide gray bars denoted ''trim'' represent low-power trim pulses. (A and B) Basic phase cycle $\phi_1 = x, y, -x, -y$; $\phi_2 = 4(-y), 4(y)$; receiver $= x, -x, x, -x$. (C) Basic phase cycle $\phi_3 = x, y, -x, -y$; receiver $= x, -x, x, -x$.

Coherence Selection Using RF-Gradients for Selective 1D NOESY

NOE spectra provide valuable information about molecular structure and can be very useful in verification of the synthetic or natural products (5, 22–24). Here, the GOESY is a virtually artifact-free, selective 1D method. As GOESY is based on B_0 gradients, it is not accessible for spectrometers lacking the field gradient accessory. Again, the RF-gradient approach can be found useful. The pulse sequence for selective 1D RFG-NOESY is shown in Fig. 1C. The combination of selective inversion and RF-gradient selection yields the 1D RFG-NOESY (1D radiofrequency gradient NOESY) spectrum with no significant artifacts, which are usual when the conventional NOE-difference method is used. To minimize the J-peaks, an extra RF-gradient was applied prior to the mixing time. Since the *selection* method is used instead of excitation sculpting, the signal loss of three-quarters must be accepted (5, 6, 25). Loss of one-half arises from the fact that only half of the magnetization can be refocused by the final gradient. Additional signal loss occurs since the magnetization of the target signal is phase encoded by the first two gradients and the 90° pulse prior to NOE mixing time creates longitudinal magnetization, which is

aligned along the $-z$ or the $+z$ axis (cosine function) due to the gradient-induced, spatially dependent phase, and so only half of the magnetization creates NOE (25). Further reduction of the signal is caused by diffusion. This is particularly problematic with small molecules in nonviscous solvents because the mixing time needed to develop NOE is often near 1 s.

The 1D RFG-NOESY sequence was tested using the same sucrose sample as for 1D RFG-TOCSY spectra. Figure 6 shows selective 1D RFG-NOESY spectra of anomeric proton recorded with different mixing times. For comparison, a slice (F2 trace of the anomeric proton) from a 2D NOESY spectrum recorded with mixing time of 1.0 s is included. The spectrum recorded with mixing time of 4 μs after the purge RF-gradient shows an antiphase J peak at 3.4 ppm. Appearance of small J peaks, although a purge RF-gradient is applied, is possibly due to J evolution during the small interpulse delays, coherence transfer induced by the z-rotation pulses, deviations in pulse angles, incomplete averaging by RF-gradients, TOCSY and ROESY effects (11), uneven excitation of multiplet by selective pulse, and imperfect 90° pulses (25). Generated antiphase magnetization is converted into zero- and double-quantum coherences by the 90° pulse prior to mixing time. If the
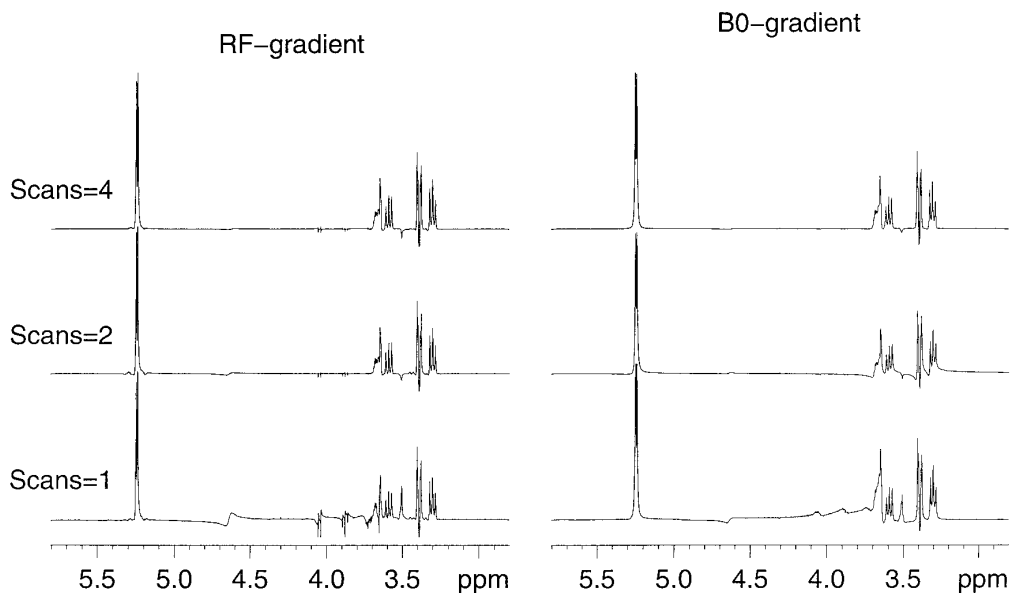
FIG. 2. The quality of anomeric proton selective 1D TOCSY spectra with 1, 2, and 4 scans. The spectra were recorded using the double-echo sequence in Fig. 1A and the corresponding sequence with B_0 gradients. The spectra were recorded on a Bruker DRX-500 spectrometer equipped with a triple-resonance probehead incorporating a single shielded gradient coil. Relaxation delay = 3.0 s, acquisition time = 1.36 s, selective 180° pulse = 20 ms Gaussian, RF-power for trim pulses and MLEV-17 = 5.48 kHz, trim-pulse length = 2.5 ms, isotropic mixing time = 152.5 ms; an exponential weighting function (0.3 Hz) was applied prior to Fourier transform. RF-gradient method: SL1 = 1.8 ms, SL2 = 2.2 ms. B_0 gradient method: gradient shape is sinusoid, gradient-pulse length = 1 ms, recovery delay = 200 μs, gradient amplitudes = 7.2 and 3.0 G/cm. The small signals at 4.05, 3.90, 3.70, and 3.50 belong to the fructose ring and are due to incoplete suppression by the RF-gradients. Similar residual signals (although smaller) can also be found in B_0-gradient-based experiments.
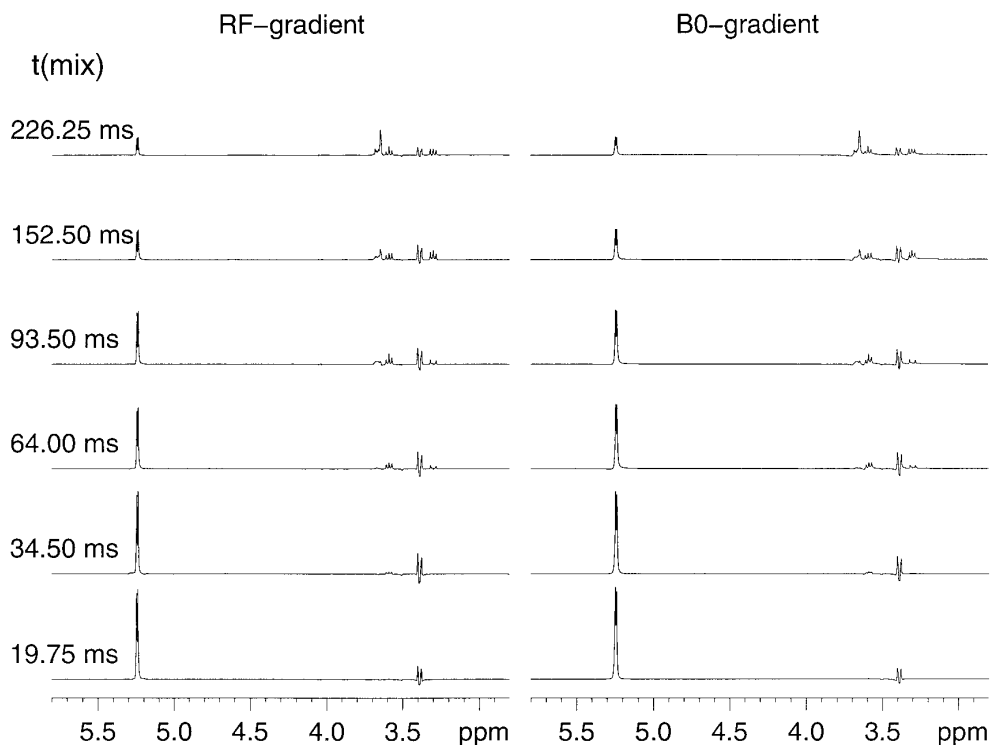

FIG. 3. Selective double-echo 1D RFG-TOCSY (sequence in Fig. 1A) and corresponding B_0 gradient TOCSY spectra of sucrose recorded with different isotropic mixing times. Number of scans was 8 for all spectra. Other parameters are the same as for spectra presented in Figure 2.
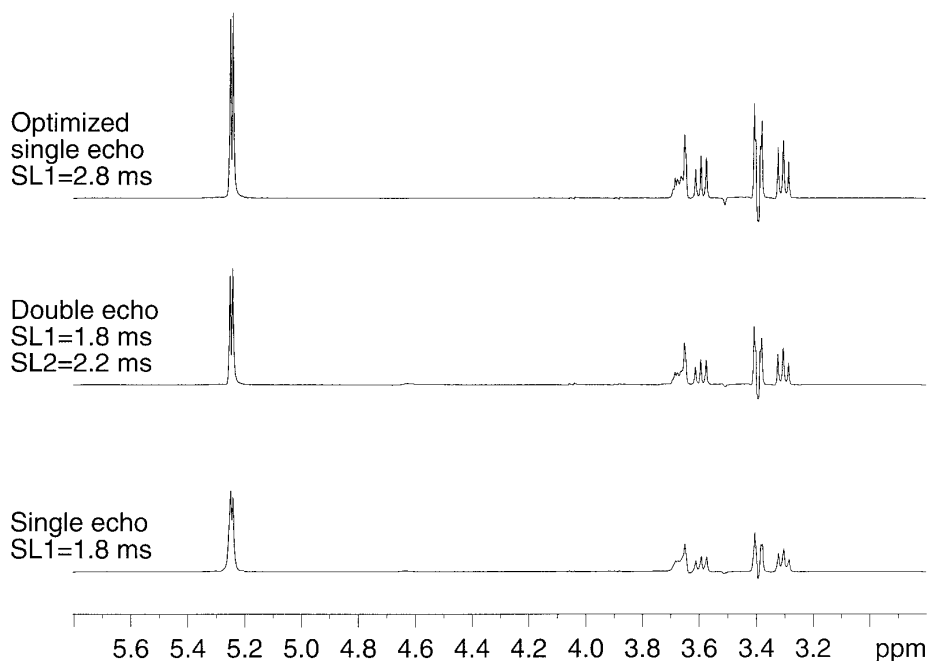
FIG. 4. Comparison of single-echo and double-echo selective 1D RFG-TOCSY spectra with different RF-gradient pulse lengths in *z*-rotation clusters. Number of scans = 8.

selective pulse excites resonance unevenly, and the 90° pulse is not perfect, so-called ''*zz*-terms'' will also be created. The zero-quantum coherence is not suppressed by the purge RF-gradient and is converted into single-quantum coherence by the NOESY read pulse. The ''*zz*-terms'' will also survive the purging, as their coherence order is zero. Due to the imperfection of the read pulse they will be converted to observable magnetization (*25*). The magnitude of the *J* peak remains constant and as NOE starts to develop the resulting in-phase NOE-peak overruns the small antiphase *J* peak when proper mixing times are used (1.0 s in this case). The spectra were recorded with 32 scans using the four step EXORCYCLE on the selective 180° Gaussian pulse. Although the four-step phase cycle is enough, increasing the number of scans improves the signal-to-noise ratio.

2D Experiments with RF-Gradient Selection

The RF-gradient selection can be easily implemented in 2D TOCSY and NOESY experiments. Magnitude mode spectra are readily obtained with a single scan per increment. This means at least fourfold savings in measurement time. The phase sensitive versions were also tested. The dephasing RF-gradient was applied after the t_1 period and refocusing with RF-gradient of same length was performed prior to acquisition. In addition, during the NOESY mixing time a purge RF-gradient was applied. The echo–antiecho method (*26, 27*) was applied to the refocusing gradient to obtain phase-sensitive spectrum (both P- and N-type spectra are recorded for one time increment). No trim pulses were applied in TOCSY. The pulse sequences for phase-sensitive, RF-gradient-selected TOCSY
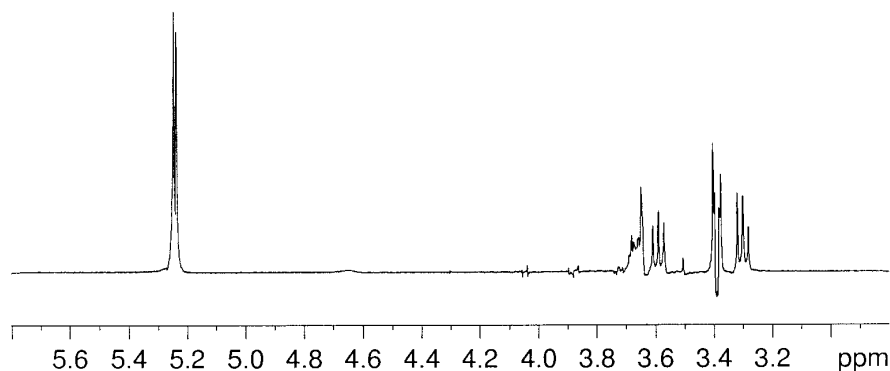

FIG. 5. The anomeric proton selective 1D RFG-TOCSY with coherence selection using RF-gradients (sequence in Fig. 1B) of sucrose. Number of scans = 2, proton 90° pulse = 16.0 μs, relaxation delay = 4.0 s, SL1 = 2.8 ms, SL2 = 5.6 ms. Other parameters are the same as for spectra in Fig. 2.
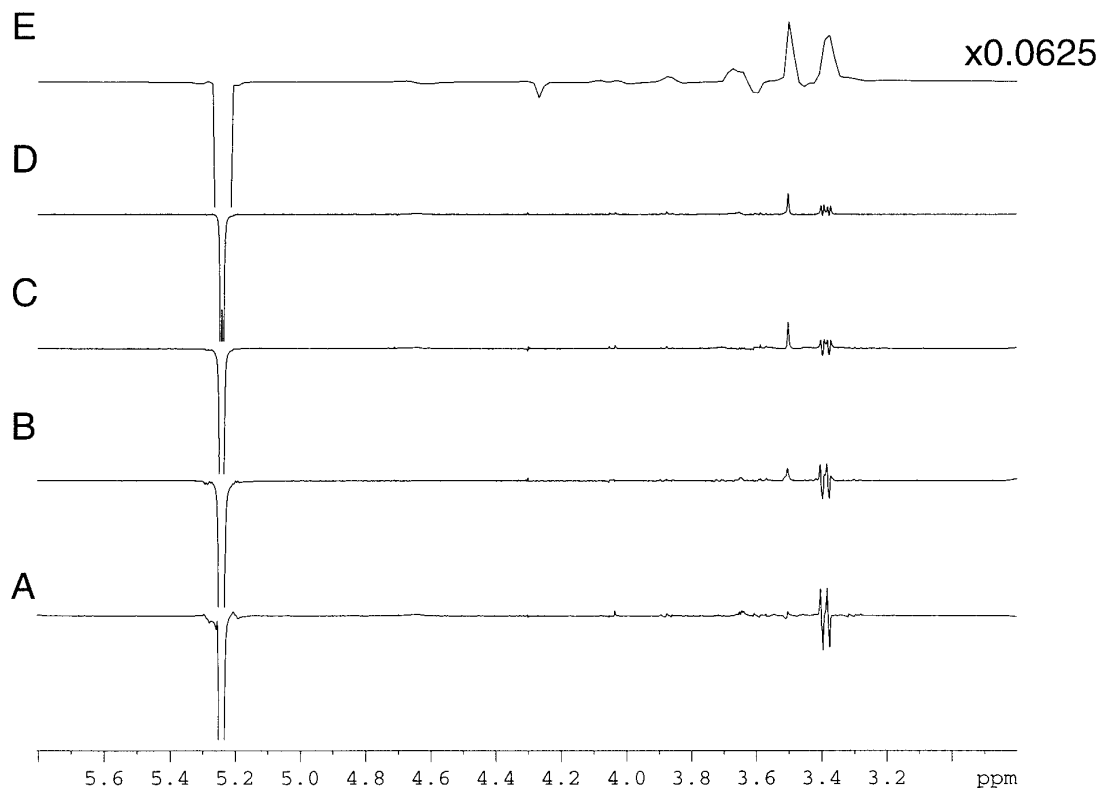
FIG. 6. Four selective 1D RFG-NOESY (sequence in Fig. 1C) spectra with different mixing times (A–D) and the anomeric proton slice of NOESY spectrum (E) of 0.5 M sucrose at 298 K. 1D spectra (A–D): Number of scans = 32, relaxation delay = 10.0 s, acquisition time = 1.36 s, selective 180° pulse = 20 ms Gaussian, SL1 = 1.4 ms, SL3 = 1.7 ms, SL2 = 2.8 ms, t_m = 4 μs (A), 125 ms (B), 500 ms (C), and 1000 ms (D); an exponential weighting function (0.3 Hz) was applied prior to Fourier transform. NOESY (E): Relaxation delay = 2.0 s, number of transients = 16, number of increments = 256, t_m = 1.0 s, resolution in f_2-dimension = 7.82 Hz/pt. The TPPI-incrementation (28) was applied to the first pulse of NOESY.

and NOESY are presented in Fig. 7. Figure 8 represents corresponding 2D RFG-TOCSY and RFG-NOESY spectra of 0.5 M sucrose in D$_2$O at 298 K. A two-step phase cycle has been used to avoid axial peaks.
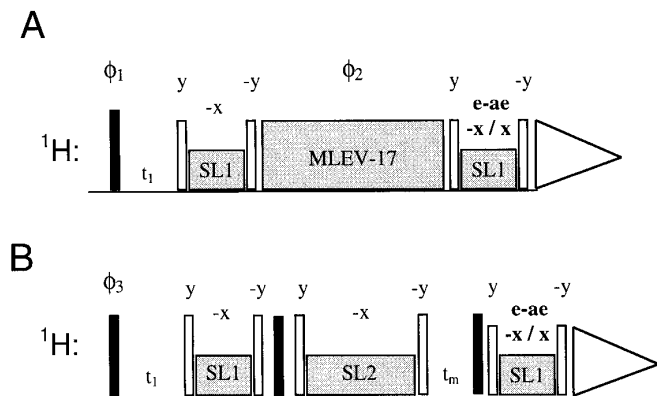

FIG. 7. Pulse sequences for phase-sensitive 2D RFG-TOCSY (A) and RFG-NOESY (B). Notation is the same as in Fig. 1. Phase-sensitive spectra are obtained by inverting the phase of the refocusing RF-gradient to record both echo- and antiecho-type spectra for the same increment (echo–antiecho method). (A) Basic phase cycle $\phi_1 = x, -x; \phi_2 = x, -x;$ receiver = $x, -x$. (B) Basic phase cycle $\phi_3 = x, -x;$ receiver = $x, -x$.

SUMMARY

In summary, RF-gradients (produced with normal ^{1}H coil) can be readily used for coherence selection/rejection. The main advantages of this method are that no hardware modifications are needed, no problems due to eddy currents are involved, no shift or coupling evolution takes place during RF-gradients, lock system can be engaged throughout the experiment, and if RF-gradients are created with long inhomogeneous pulses implemented in z-rotation composites, all homonuclear B$_0$-gradient-based experiments can be easily converted into RF-gradient-based ones by simply replacing the B_0 gradients with z-rotations. As a drawback, effective RF-gradients produced with a normal coil and proton transmitter tend to be somewhat longer than conventional B$_0$-gradient pulses. This is not usually a problem with small- and medium-sized molecules, as T_2 relaxation times are long. However, with large molecules (i.e., short transverse relaxation times) increasing the duration of pulse sequence might decrease the signal intensity.

The RF-gradient methods should not be considered as an alternative to B_0 gradients, but rather as a substitute for those spectrometers lacking the field gradients. Simultaneous use of B_0 and RF-gradients would be an interesting possibility to
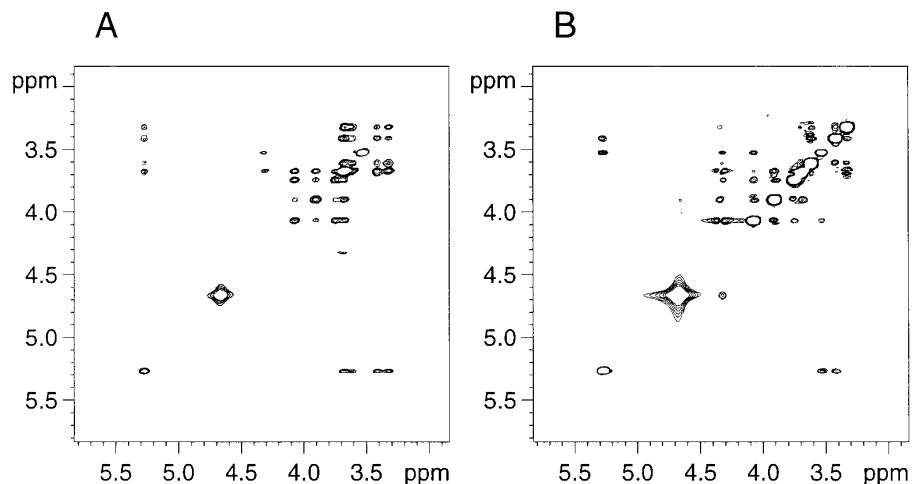
FIG. 8. 2D RFG-TOCSY (A) and RFG-NOESY (B) spectra of 0.5 M sucrose in D_2O at 298 K recorded using sequences presented in Fig. 6. Experimental parameters: Bruker DRX-500 NMR spectrometer equipped with Bruker triple-resonance probe and z-axis gradient system, 500 MHz ^{1}H frequency, relaxation delay = 4.0 s, $t_{1max} = t_2 = 85.2$ ms, number of transients = 2, number of time increments = 256, number of sampled points = 256, (A) SL1 = 1.8 ms, t_{mix} = 147.4 ms, RF-power for MLEV-17 = 5.48 kHz, (B) SL1 = 1.8 ms, SL2 (spoil) = 1.4 ms, t_m = 1.0 s. The t_1 and t_2 domains were zero-filled once, and both dimensions were multiplied with squared cosine function prior to Fourier transformation.

further improve gradient selection and to avoid the shift or coupling evolution during gradient pulses.

EXPERIMENTAL

All spectra were recorded on a Bruker DRX-500 NMR spectrometer (500 MHz ^{1}H frequency) equipped with Bruker triple-resonance probe and z-axis gradient system at 298 K. The length of the 90° pulse on high power level was 5.6 μs (16.0 μs for the spectrum in Fig. 5). Thus, during the spin-lock pulses (durations: 1–3 ms), a proton on resonance undergoes 45–134 rotations. The probe inhomogeneity was measured with successive rotations to give intensity ratios of 1.00:0.89: 0.79:0.70 with 90°, 450°, 810°, and 1170° pulses, respectively. The 0.5 M sucrose sample was prepared by dissolving sucrose (not D-exchanged) into 0.7 ml of 99.5% D_2O.

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