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Communication

Removal of *J*-coupling peak distortion in PGSE experiments

Allan M. Torres *, Ruby Dela Cruz, William S. Price

Nanoscale Organisation and Dynamics Group, College of Health and Science, University of Western Sydney, Penrith South DC, NSW 1797, Australia

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ABSTRACT

Peak distortion caused by homonuclear *J*-coupling is a major problem that limits the utility of the pulsed-field gradient spin–echo (PGSE) method for studying translational diffusion. This unwanted effect can be removed by incorporation of anti-phase magnetization purging pulse elements at the end of the spin–echo sequence. Three methods, namely, trim-pulse, homospoil pulse gradient and chirp based *z*-filter were evaluated as potential candidates for an improved NMR diffusion method that is less sensitive to *J*-coupling peak distortion. The chirp based *z*-filter was found to be excellent in suppressing anti-phase magnetization while leaving the in-phase magnetization basically intact in spin–echo and stimulated-echo based experiments. The incorporation of chirp based *z*-filter into PGSE could allow diffusion analysis that would otherwise be impossible by conventional means.

1. Introduction

The pulsed gradient spin–echo (PGSE) [1] and the pulsed gradient stimulated-echo (PGSTE) [2] experiments are widely used methods in nuclear magnetic resonance spectroscopy (NMR) to study diffusion of molecules and ions in different solvent environments [3–8]. Such experiments, especially when the results are displayed in a two-dimensional format, are often referred to as diffusion ordered spectroscopy (DOSY) [8]. Knowledge about the diffusive properties of chemical entities is important as random translational motion is the main form of transport in biological and chemical systems and is a limiting factor for many chemical reactions [6,9]. Consequently, diffusion measurements can be utilized to examine intermolecular interactions, binding and self-association.

As in many analytical techniques, precise and accurate measurements in PGSE experiments are vital for correct interpretation of data because even small inaccuracies could lead to erroneous conclusions. Peak distortion caused by proton homonuclear *J*-coupling is a big problem [10,11] in the Hahn-based PGSE and is one of the major factors that complicates the analysis of this useful experiment. This *J*-coupling effect leads to unwanted mixtures of in-phase and anti-phase magnetizations created during spin–echo delay; the latter is particularly undesirable as it complicates peak integration. Moreover, as the anti-phase magnetization is usually 90° out-of-phase with the in-phase magnetization, peak analysis becomes very difficult since the peak appears as a dispersion signal creating a large baseline shift. The presence of this type of peak also complicates analysis for samples containing mixture of biomolecules where peak overlap due to the presence of many proton signals is common.

Efforts have been made to address the unwanted effects of homonuclear *J*-coupling in NMR diffusion experiments; these include proper choice of delays and the use of other methods such as PGSTE [2], a variant of PGSTE that includes a spin-lock [12], and oscillating gradient spin echo (OGSE) [10,11,13,14]. Processing the spectra in absolute value mode can be useful in some circumstances but this is no solution as it (i) does not remove the problem, (ii) broadens peaks and (iii) makes the noise positive. The approach we are considering here is the use of NMR "purge" elements that can remove anti-phase magnetizations without severely affecting in-phase magnetizations. Among the popular methods for the removal of anti-phase magnetizations is the trim-pulse or spin-lock where a weak continuous RF field is applied for a short time along the axis of the in-phase magnetization so it is unaffected while anti-phase magnetization, which is usually 90° out-of-phase, is randomized [12,15]. Homospoil gradient pulses have also been successfully used to remove magnetization from spin-coupled systems in PGSE edited experiments [16,17]. And more recently, an improved *z*-filter that employs a chirp pulse [18,19] and homospoil gradient pulses has shown impressive performance in removing distorted peaks in many common 2D experiments [20,21]. In this communication, we investigate the performance of these three techniques in

* Corresponding author. Fax: +612 4620 3025.
E-mail address: a.torres@uws.edu.au (A.M. Torres).

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removing anti-phase magnetization in PGSE experiments (both Hahn and stimulated-echo based sequences). The inclusion of anti-phase magnetization purging elements results in an improved robust NMR diffusion experiment that is applicable to a wide range of samples especially those containing a mixture of compounds with overlapping peaks.

2. Experimental

Experiments were performed on a Bruker Avance 400 spectrometer equipped with 5 mm BBO probe with single axis *z*-gradient capable of achieving a maximum gradient strength of 53.5 G cm^{-1}. In all experiments, the probe temperature was 298 K, the ^{1}H 90° pulse length was ~14 μs and number of scans equal to four. The trim-pulse employed had an RF field strength of 10 kHz and a duration τ_m = 2 ms. The homospoil gradient applied in all cases had a strength of 2.7 G cm^{-1} and a duration τ_h = 10 ms. The chirp pulse, which was calibrated according to the recommendation of Thrippleton and Keeler [20], has an RF field strength of 1 kHz, duration τ_c = 30 ms and was swept through 20 kHz. Diffusion experiments on the mixture amino acid D-Leu (Sigma–Aldrich, St. Louis, MO) and hexapeptide ImFSRS (chemically synthesized according to procedure described by Bansal et al. [22]) was performed with Δ = 30 ms ($2\tau \approx \Delta$) and δ = 3.5 ms. A series of 1D diffusion spectra was obtained by incrementing the gradient strength from 0% to 90% of the maximum power in 18 steps.

3. Results and discussion

3.1. The PGSE pulse sequences and 2-propanol test sample

The pulse sequences for the standard and three modified PGSE sequences that incorporate the anti-phase purging elements spin-lock, homospoil gradient, and chirp with homospoil gradient are shown in Fig. 1. To better evaluate the effectiveness of these purging techniques in various *J*-coupling situations, spin–echo versions of the above sequences (without the gradient pulses during the τ delays) were first performed on a test sample containing 10% (v/v) 2-propanol in D_2O. A series of 1D spectra was acquired with each method wherein τ was increased from 0 to 1/2*J* in 1/8*J* increments where *J* = 6.24 Hz for the CH_3 and CH protons of the test molecule.

3.2. The standard spin–echo

A series of spectra of 2-propanol for the standard spin–echo and modified sequences is shown in Fig. 2. The *J*-coupling problem in spin–echo type experiments is clearly demonstrated in the spectra acquired with the standard sequence. The spectrum for τ = 0 was comparable to a normal 1D spectrum where all peaks are in-phase. Similarly, the spectrum at τ = 1/2*J* resulted in two peaks that were completely in-phase, although in this case the CH_3 doublet was negative. Note that these two choices of τ are acceptable for diffusion peak analysis since no anti-phase peaks are expected, however for real samples containing more than one *J*-coupling constant, it would be difficult, or even impossible, to find a value that would result in all peaks being in-phase without the sequence being too long and losing too much signal to relaxation. Setting τ to 1/4*J* led to pure anti-phase peaks which were actually 90° out-of-phase with the in-phase peaks; these are actually dispersion signals containing positive and negative lobes that are unwanted as they are difficult to integrate. The purging schemes being investigated here would attempt to remove these unwanted magnetizations so that only in-phase magnetization will be obtained. Setting τ to 1/8*J* or 3/8*J* resulted in equal mixture of in-phase and anti-phase components

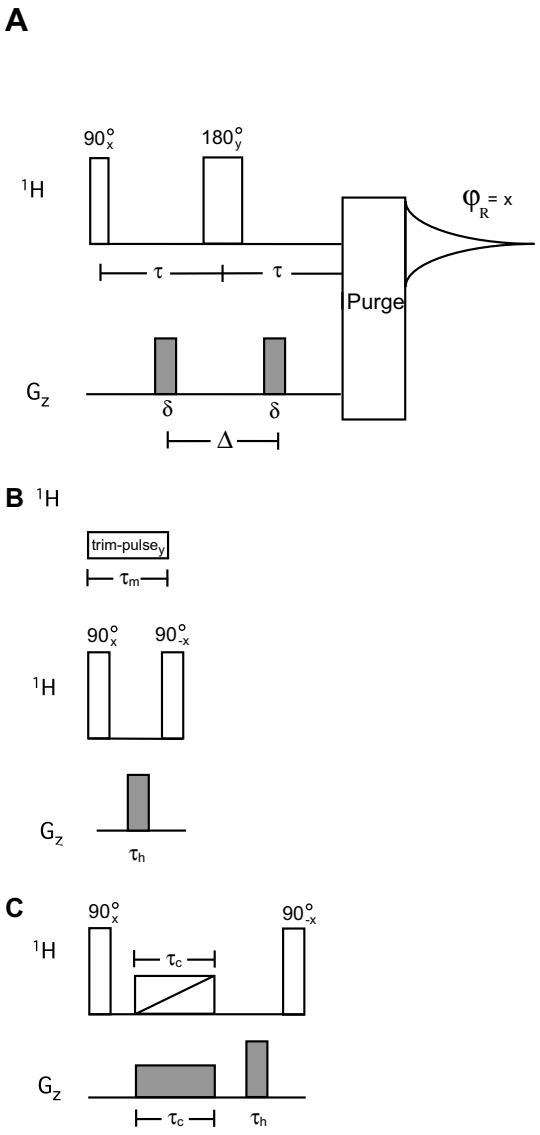

Fig. 1. Pulse sequences for the standard PGSE and modified PGSE experiment designed to remove anti-phase magnetizations. The standard sequence does not incorporate the purge element of the modified sequences. Purge element: (A) trim-pulse or spin-lock, (B) homospoil gradient, (C) chirp based *z*-filter. Homonuclear *J*-coupling occurs during the spin–echo delay τ. The duration of the two magnetic field gradient pulses δ and their separation Δ are important in obtaining diffusion coefficients.

which were more difficult to analyze than when τ was set to 1/4*J*. These two situations represent what typically occurs in many PGSE experiments where the presence of dispersive anti-phase peaks causes undesirable modulation of peak lineshapes.

3.3. Trim-pulse sequence

Application of a spin-lock or trim-pulse for 2 ms resulted in dramatic improvement over the standard method especially for the CH_3 doublet of 2-propanol (Fig. 2C). Although the spin-lock spectra at τ = 0 and 1/2*J* where no anti-phase peaks are anticipated were similar to those of the standard spectra, those for other cases were significantly better. The CH_3 peaks in the trim-pulse spectra at τ = 1/8*J* and 3/8*J* were homogeneously in-phase and thus the anti-phase dispersion peaks were effectively removed. The trim-pulse purging method spectra however displayed some undesirable properties in peak lineshape. Although the CH_3 peak at 1/4*J*

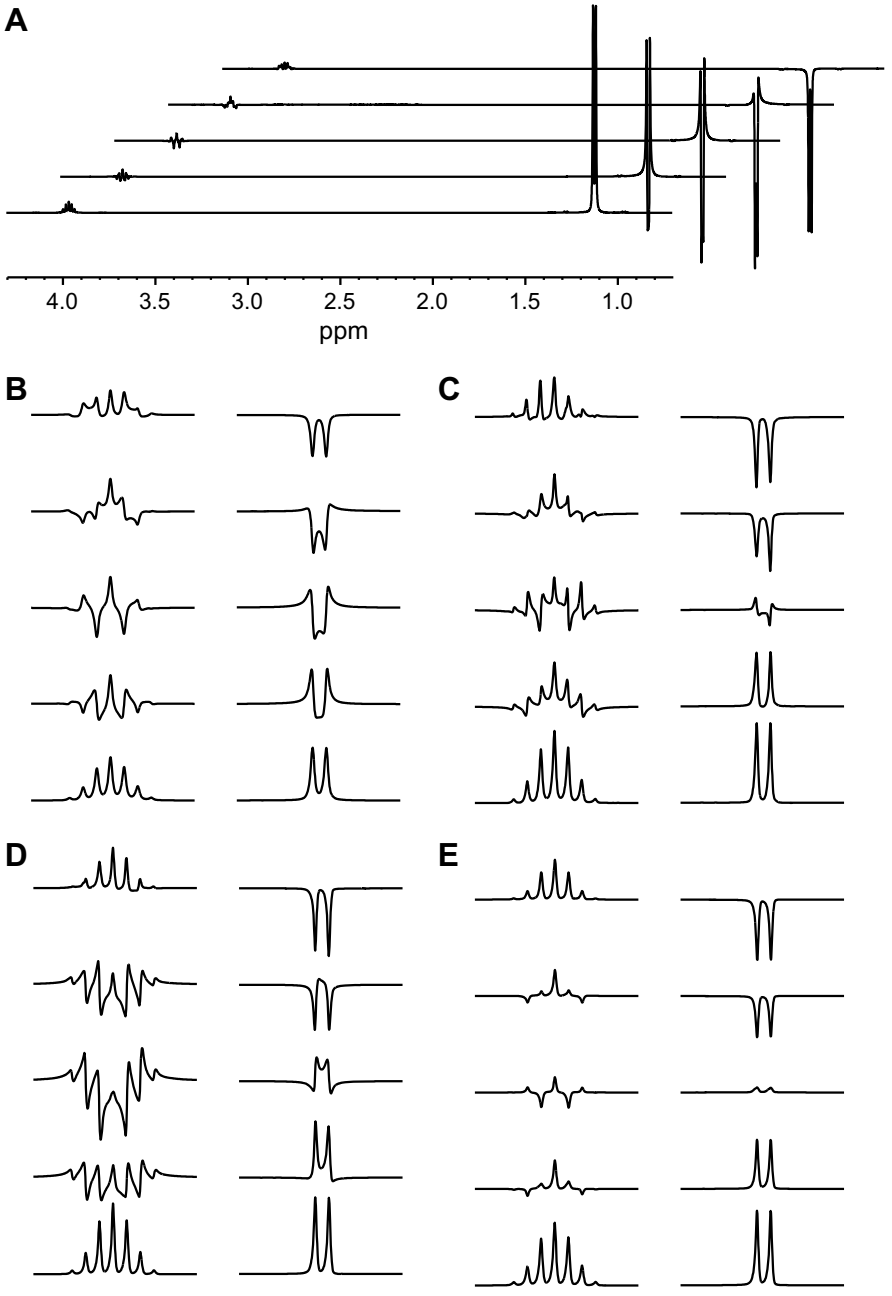

Fig. 2. Performance of the standard and modified PGSE on 10% (v/v) 2-propanol sample in D_2O at 298 K in the absence of gradient pulses. A series of five spectra are plotted (from top to bottom) with decreasing τ values from $1/2J$ to 0 in $1/8J$ increments where J is the homonuclear spin–spin coupling in Hz. (A) standard PGSE spectra showing the multiplet near 4.0 ppm and doublet near 1.0 ppm. Expanded peaks regions for (B) standard PGSE and modified PGSE containing (C) trim-pulse, (D) homospoil gradient, (E) chirp based z-filter.

was significantly smaller than that in the regular method, it was somewhat distorted. Some distortion was also evident for the in-phase peaks at $3/8J$ wherein the doublet peak was unsymmetrical. There were some improvements in the CH multiplet in the trim-pulse spectra but obviously some distortions were still evident.

3.4. Homospoil gradient sequence

Like the regular and trim-pulse sequences, the CH_3 peaks at $\tau = 0$ for the sequence that incorporates a homospoil gradient were undistorted appearing as completely in-phase (Fig. 2D). For other τ values, the anti-phase peak suppression was clearly evident however there were still obvious traces of dispersive type peaks. For $\tau = 1/4J$, for example, the CH_3 peak appeared more as anti-phase dispersion than in-phase absorption. It can be stated therefore that homospoil gradient sequence provided dramatic suppression of the anti-phase peaks but its efficiency was somewhat lower than the trim-pulse method.

The inefficiency of the homospoil method in suppressing the anti-phase peaks may be attributed to its known inability to effectively suppress zero-quantum coherences. The homospoil sequence works by first converting the desired in-phase magnetization (generated by the first 90° RF pulse element) to longitudinal z-magnetization; the undesired anti-phase magnetization meanwhile is converted into zero-quantum and multiple quantum coherences. Application of the gradient pulse or homospoil destroys any coherences greater than zero but leaves longitudinal magnetization and zero-quantum coherences intact. The second

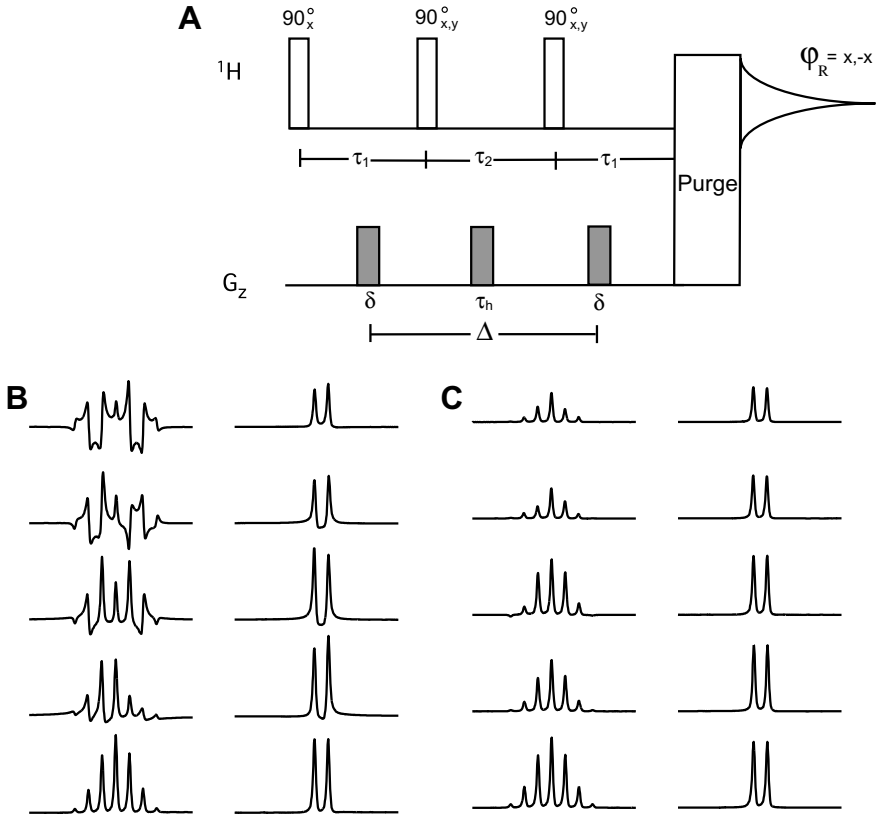

Fig. 3. Application of chirp based *z*-filter to PGSTE. (A) Pulse sequence for PGSTE (without purge) and modified PGSTE incorporating anti-phase purge element (with purge). Experiments are performed with at least two scans wherein the phase of the last two 90° pulses is incremented by 90° while the receiver phase is alternated. (B and C) Performance of the standard PGSTE (B) and modified PGSTE with chirp based *z*-filter (C) on 10% (v/v) 2-propanol sample in D_2O at 298 K. Experiments were performed similar to that in Fig. 2.

90° RF pulse converts the desired longitudinal magnetization into transverse in-phase magnetization however the zero-quantum coherences are also converted back into unwanted anti-phase magnetization which causes peak distortion.

3.5. Chirp based z-filter

The chirp with homospoil gradient provided the most impressive anti-phase peak suppression in PGSE (Fig. 2E). In all cases, the CH_3 doublet appeared in-phase with no trace of anti-phase characteristics. The peaks were also symmetrical in contrast to those acquired with the trim-pulse and homospoil gradient methods. Comparison of the chirp based *z*-filter and homospoil pulse sequences as shown in Fig. 1, shows that they are somewhat similar and that their differences are due the presence of the chirp 180° adiabatic pulse and the homospoil gradient pulse that is applied simultaneously. These two elements are actually designed to remove the zero-quantum coherences and non-zero level coherences that can lead to anti-phase peaks. Details of its mechanics are described elsewhere [20]. It is clear that the chirp and first gradient pulse are responsible for the superb anti-phase suppression of this purging method. The second homospoil gradient pulse ensures that other unwanted coherences are effectively suppressed. The disadvantage of this over the other two methods is that the duration of the chirp pulse of (30 ms) is considerably longer than the trim-pulse (2 ms) and the homospoil gradient (10 ms).

3.6. PGSTE and modified PGSTE with chirp based z-filter

PGSTE can also benefit from purging sequences considered here, especially the chirp based *z*-filter. This stimulated-echo based experiment is preferred over Hahn-based PGSE in studying diffusion of molecules with short T_2 relaxation values such as large biomolecules like proteins and polypeptides. Note however that the Hahn-based experiments can be preferable over PGSTE in drug binding studies since they are more effective in removing resonances from large molecules (due to their short T_2)—thus making the drug resonances easier to observe [23,24]. As mentioned in Section 1, PGSTE can be used to address unwanted effects of *J*-coupling and this is because this method converts the desired magnetization partly to longitudinal *z*-magnetization which is invariant to homonuclear *J*-coupling. The PGSTE sequence as shown in Fig. 3A contains two different delay periods, τ_1 and τ_2 during which relevant magnetization evolved. During τ_1, the magnetization exists as transverse magnetization which may be susceptible to homonuclear *J*-coupling but during τ_2, the magnetization exists as longitudinal *z*-magnetization. Therefore, to lessen the effect of *J*-coupling in PGSTE, the delay τ_1 (related to δ) should be set as short as possible while τ_2 can be varied accordingly. Note however that there will always be peak distortions in PGSTE as τ_1 has finite values normally in the order of milliseconds.

The effects of varying delay τ_1 in the diffusion spectra of the 2-propanol sample obtained using standard and modified PGSTE with chirp based *z*-filter are presented in Fig. 3B and C, respectively. The series of spectra were acquired similar to that earlier for Hahn-based PGSE (Fig. 2) where $\delta = 0$ and the τ_1 delay varied by $1/4J$. In the standard PGSTE spectra in Fig. 3B, significant peak distortions were evident for $\tau_1 > 0$ and the distortion profiles vary with τ_1. In contrast, in the modified PGSTE spectra, peak distortions were basically eliminated by the application of the chirp based *z*-filter purging sequence at any τ_1 value. These results therefore clearly demonstrate the applicability of this particular purging

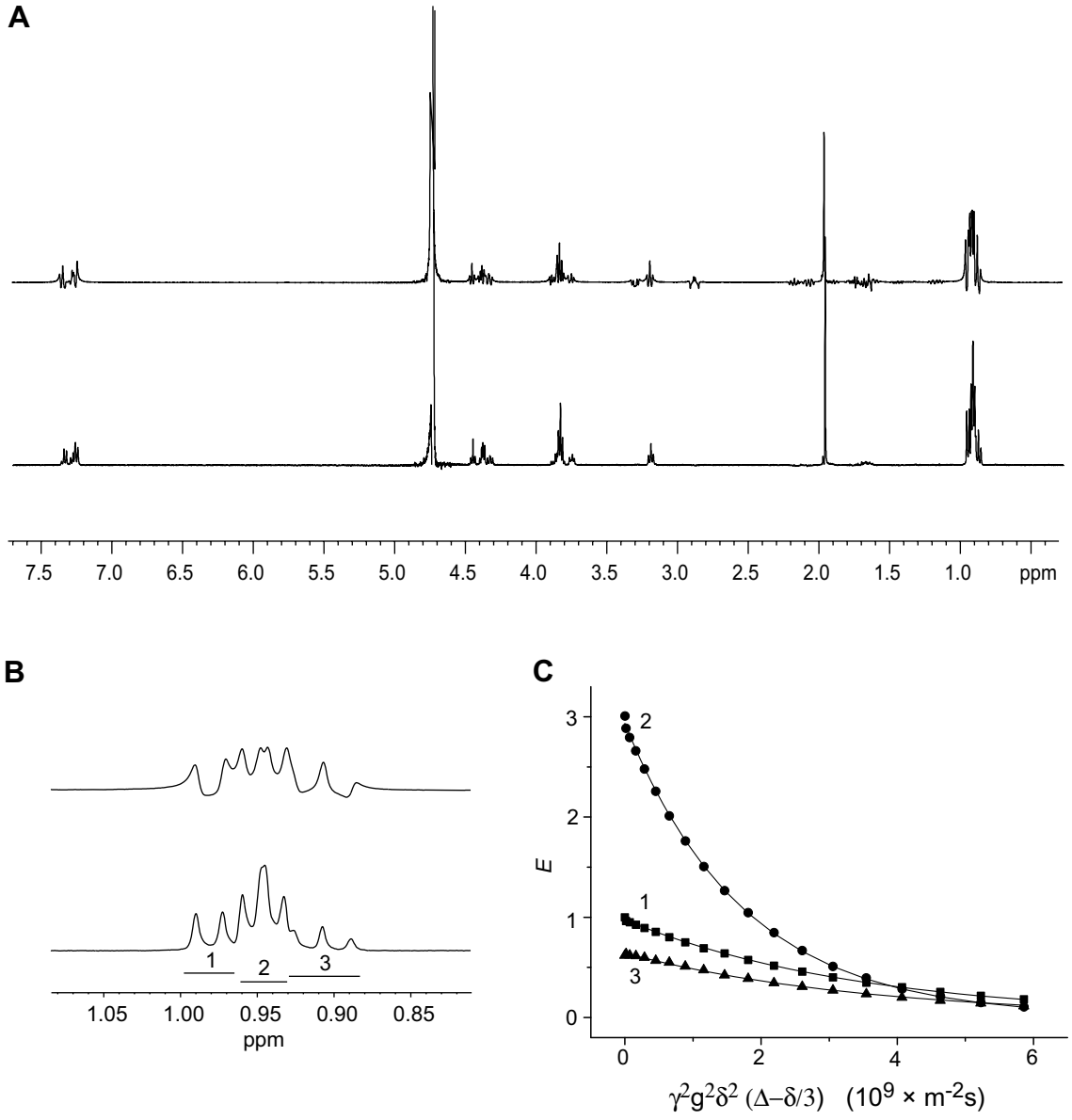

Fig. 4. Comparison of the standard and modified PGSE with chirp based *z*-filter. (A) Standard (top) and modified (bottom) PGSE spectra of sample containing an equimolar mixture of an amino acid (D-Leu) and a hexapeptide (ImFSRS). (B) Methyl region of the two spectra. Numbered regions (1,2,3) indicate peaks that were distinguishable in the modified spectra which were subsequently integrated in PGSE experiment. (C) Diffusion plots of the integrated peaks in the modified spectra showing similar decay rates for peaks 1 and 3 and significantly faster decay for peak 2.

sequence to PGSTE. Similarly, the other purging sequences tested earlier on PGSE could also be expected to decrease the peak distortions in PGSTE.

3.7. ImFSRS and D-Leu

As mentioned earlier, the utility of the purging sequences in PGSE may be best realized on samples containing mixtures of species with different diffusion properties that is characterized by overlapping peaks. Spectra obtained using standard PGSE and PGSE with chirp based zero-quantum purge sequences for a sample containing an amino acid (D-Leu) and a hexapeptide (ImFSRS where m is a D-methionine) are shown in Fig. 4A. The regular PGSE spectrum showed many distorted peaks while the spectrum obtained with purged PGSE was much cleaner as the peaks are generally in-phase. An expanded view of the CH_3 peak region of the standard experiment revealed that it is difficult to distinguish individual multiplet structures so that it is not possible to perform peak integration (Fig. 4B, top). The purged experiment (Fig. 4B, bottom), on the other hand, resembles a regular 1D pulse and acquire spectrum as the three multiplets (one doublet and two triplets) are discernible. It is therefore possible to quantify each peak by integration. The diffusion plots in Fig. 4C show excellent correlation of data for each peak multiplet. Non-linear least squares analysis yields diffusion coefficients of $2.90 \times 10^{-10}\ m^2\ s^{-1}$ for doublet on the left, $5.73 \times 10^{-10}\ m^2\ s^{-1}$ for the triplet on the middle, and $2.82 \times 10^{-10}\ m^2\ s^{-1}$ for the remaining triplet on the right. Clearly, the middle triplet belongs to the lower-molecular weight species (D-Leu) as its diffusion coefficient was much higher than the other two values which can be attributed to the hexapeptide.

It has been shown that cascading the chirp *z*-filter sequences bring about better suppression ability [21]. As shown in this study, this is not necessary as the suppression was already sufficient. The downside to this method is that its duration is considerably longer.

It may also be possible to include a trim-pulse at prior to or at the end of the chirp sequence but our results showed little benefit from such approach.

4. Conclusion

We have shown here that chirp based *z*-filter is an excellent method for selecting in-phase magnetization in diffusion experiments such as PGSE and PGSTE. This method should be applicable to many spin–echo based methods where *J*-coupling peak distortions are unwanted. It should also find utility in analysis of biomolecules where peak overlap is common. It may be possible to use this technique for spectral editing or as an additional experiment to confirm or complement results obtained using standard spin–echo based experiments.

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