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Self-Compensating Pulsed Magnetic-Field Gradients for Short Recovery Times

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The use of pulsed magnetic field gradients (PFG) in high resolution NMR has recently attracted much attention (1-16). This break trough was possible due to the availability of shielded gradient coils which improved the performance of PFG's dramatically and resulted in a substantial reduction of the recovery time needed to reach a stable homogeneous static magnetic field after the application of a gradient. Many of the new applications of PFGs in high resolution NMR are designed to reduce the phase cycling needed to define the coherence pathway and to cancel artifacts. Gradients up to 30 G/cm are usually more than sufficient for these experiments. For stronger gradients or without shielded gradient coils severely distorted NMR signals may still be observed unless long recovery times are used. When we wanted to study exchange times of labile protons and water molecules in the interior of proteins by differences in the respective diffusion times it was indicated to use the highest possible gradient strengths with the shortest recovery times possible to prevent substantial exchange during the application of the gradient and the dead time. A gradient strength of up to 180 G/cm was available but the dead time of a few milliseconds was too long compared to the expected exchange time of less than one millisecond. Due to the fast exchange no difference in the diffusion constants could be measured. Similar measurements were recently proposed (17A) using a moderate PFG strength demonstrating the principle but no evaluation of the exchange times was given.

If gradients with long recovery times are applied to transverse magnetization relaxation and exchange can lead to substantial signal loss. As an alternative to long waiting times a preemphasis can be used. This unit shapes the gradient pulses, e.g. with exponentials with different time constants and amplitudes, which leads to a compensation of the transient response induced in the system when switching on or off magnetic field gradients. The adjustment requires manipulations which are similar to shimming a magnet and may require substantial time and experience. The induced transient signals will usually have a complex spatial and time dependence and can not fully be corrected by a preemphasis unit.

In this communication we propose a PFG sequence which is self compensating and allows to greatly reduce transient responses without tedious adjustments and without the need of any additional hard-

ware. Successive PFGs are very reproducible. The application of a sign inverted but otherwise identical PFG will result in transient responses which are also opposite in sign. Two PFGs back to back which have different sign will therefore tend to compensate each others imperfections. In order not to refocus the effect which the first gradient has on the spins a 180 degree rf pulse has to be applied between the two gradients. In this way the location dependent phase of the individual magnetization components obtained during the first gradient will further develop during the second gradient. Fig. 1A and B shows the implementation into a pulse sequence with which the recovery time after the application of a gradient can be measured. The gradient with length $2\tau_g$ is split up in two gradients with opposite sign and length τ_g which are separated by a 180 degree radio frequency pulse. Fig 1C and D show the implementation of this PFG sandwich into a pulse sequence for diffusion measurements. A similar pulse sequence was proposed earlier to compensate for strong background magnetic field gradients in solids (18A). Here we are not concerned about static background gradients which are negligible in high resolution NMR, but we want to compensate transient responses which are due to the application of strong gradients.

Fig. 2 compares ^1H NMR spectra of a 20 mM solution of the protein bovine pancreatic trypsin inhibitor (BPTI) in 90% H_2O / 10% D_2O, which were recorded, respectively, with conventional PFGs (A, C), or with the newly proposed self compensating PFGs (B, D). The experimental conditions for A and B (Fig. 1A and B) on the one hand, as well as for C and D (Fig. 1C and D) on the other hand were otherwise identical. The measurements were performed on a BRUKER AMX500 NMR spectrometer equipped with shielded gradient coils and a 10A gradient amplifier, no preemphasis was used and the lock signal was not blanked during the application of the gradients. The PFGs applied to obtain the spectra had a strength of 60 G/cm and a modified rectangular shape where the last quarter of the rectangle was multiplied by a cosine squared function (Fig. 1). The length was 2ms for a single gradient and 1ms each for the self compensating gradients. The comparison of the recovery times (Fig. 2A and 2B) were measured with only 4 microseconds delay between switching off the magnetic field gradient and the radio frequency excitation pulse and subsequent detection of the FID. Between the gradients and the 180 degree pulse in Fig. 2B a delay of 4 μs was used. The improved recovery

time performance in the experiment of Fig. 2B demonstrates the potential of self compensating PFGs. If more than one gradient is applied in the same pulse sequence, in our example for diffusion measurements, even more dramatic differences can be observed. The spectra shown in Fig. 2C and 2D were measured using the pulse sequence of Fig. 1C and Fig. 1D, respectively. The delays chosen were τ_1=32 μs, τ_2=4 μs, τ_3=24 μs. The spectrum in Fig. 2D and the absence of an interpretable spectrum in Fig. 2C are an impressive illustration of the performance of self compensating PFGs.

The relative improvements do not depend on the special hardware configuration chosen. Measurements with different gradient shapes, with and without preemphasis, as well as using the z-shim coil to deliver the gradients confirm the general applicability of the technique. The recovery time increases when unshielded coils (e.g. z-shim) are used or for rectangular shapes, whereas it gets shorter when using a preemphasis unit. The 180 degree pulse will usually lead to some signal loss due to its radiofrequency inhomogeneity. It depends on the particular experiment if the transverse relaxation during a long recovery delay or the 180 degree pulse will result in the smaller sensitivity. If the magnetization of interest is in a z state the length of the recovery time is often not so critical if the longitudinal relaxation is long. But even in this case short recovery may be necessary, e.g. if chemical exchange during the recovery delay is of concern.

In Fig. 3 two self diffusion measurements are presented for H_2O in a 20 mM solution of BPTI in 90% H_2O / 10% D_2O, which were recorded, respectively, with conventional PFGs (Fig. 1C), or self compensating PFGs as shown in Fig. 1D. For the PFGs the same shapes as for Fig. 2 were used. The measurement was done using constant time intervals and increasing the gradient strength from 18 to 180 G/cm using a BRUKER 30A gradient amplifier without a preemphasis. The delays τ_1 and τ_2 were chosen 1ms which made it possible to integrate the spectra taken with the conventional PFGs for small gradient powers. For gradient strength larger than 120 G/cm the spectrum resulting from sequence of Fig. 1C was so distorted that an integration of the water resonance was no longer possible, actually negative intensities would have resulted. For a rectangular gradient shape and the sequence of Fig. 1C the formula $\ln(S/S_0)=-4\gamma\tau_r^2 g_z^2(5\tau_r/3+\tau_1)D$ describes the signal attenuation due to diffusion (17),

where D is the diffusion constant and g_z the gradient strength. The straight line through the data points represents a fit of a linear function to $\ln(S/S_0)$. For a general shape the gradient has to be integrated over time which results in a more complicated dependence on the gradient length τ_r and the time between the gradients τ_1 (17, 18). Instead of calculating the actual dependence for the shape used in our measurement we determined the known self diffusion constant of H_2O in 90% H_2O / 10% D_2O (19) with the method of Fig. 1D and used it to calibrate the measurements of Fig. 3. The self diffusion constant for H_2O in 20 mM BPTI determined in this way is 1.0×10^{-5} cm^2/sec. The curved line is no fit and should only guide the eye.

In conclusion, the results obtained with the experimental scheme of Fig. 1 demonstrates that the application of a inverted repeat of PFGs for reduction of unwanted transient distortions during the switching on and off times enables much shorter recovery times than would be possible with one single magnetic field gradients. This result is independent of a special hardware configuration and promises to yield spectra of generally improved quality, which will be much less affected by transient response.

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FIGURE CAPTIONS

Fig. 1

Pulse sequences with pulsed magnetic field gradients (PFG) using the conventional and the newly proposed sandwich arrangement of PFG's. rf stands for radiofrequency and g_z for a magnetic field gradient (PFG). The vertical bars represent radio frequency pulses, where the different pulse lengths for the $\pi/2$- and the π-pulses are distinguished by the width of the bars. The gradient pulses are indicated by the grey shapes. The time period τ_g indicates the duration of a PFG. The delays between the gradients and between the gradients and the radiofrequency pulses are typically a few microseconds (see Fig. 2). The acquisition times are denominated by *t*. (A) Pulse sequence for measuring the recovery time after the application of a PFG. (B) the same as (A) but with self compensating PFG's. (C) Pulse sequence used for the diffusion measurements. (D) the same as (C) but with self compensating PFG's.

Fig.2

^{1}H NMR spectra recorded with a 20 mM solution of BPTI in 90% H_2O / 10% D_2O at pH 4.6 and T = 293 K using the pulse sequences indicated in Fig. 1. From the original FID its time average was subtracted to reduce the residual H_2O signal (20), averaging was done over 50 complex points. No baseline correction was used. All spectra are plotted with identical noise levels. All gradient pulses had a length of 2ms for single PFGs and of 1ms each for sandwich PFGs. The individual gradient pulses had a modified rectangular shape were the last quarter of the shape was attenuated as cosine squared. The recovery time used after the gradients was 4 microseconds. The gradient strengths was 60 G/cm for all gradients. (A) Spectrum acquired using the conventional pulse sequence shown in Fig. 1A with 4 microseconds delay between switching off the gradient and the 90 degree excitation pulse. (B) the same as (A) using the pulse sequence with self compensating PFG's (Fig. 1B), with 4 microseconds delay between rf pulses

and gradients. (C) Conventional diffusion measurement sequence (Fig. 1C) with τ_1=32 microseconds and τ_2=4 μs. (D) The same as (C) using the pulse sequence given in Fig. 1D with τ_1=32 μs, τ_2=4 μs and τ_3=24 μs.

Fig. 3

Self diffusion measurements of H_2O in 90% H_2O / 10% D_2O and 20Mm BPTI at pH 4.6 and T = 277 K using the pulse sequences indicated in Fig. 1C (●) and Fig 1D (○). Gradients in Fig. 1C were 2ms each and τ_1=τ_2=1 ms. For Fig 1D all gradients had a length of 1 ms and τ_1=τ_2= 1ms and τ_3= 24 μs. All gradient pulses had a modified rectangular shape were the last quarter of the shape was attenuated as cosine squared. To determine the self diffusion constant different experiments were done with gradient strengths 18, 36, 54, 72, 90, 108, 126, 144, 126, 180 G/cm. The straight line through the data points represents a fit of a liner function to $\ln(S/S_0)$. The self diffusion constant D for H_2O in 20 mM BPTI determined (see text) is $1.0 \times 10^{-5} cm^2/sec$. The curved line is just drawn to guide the eye and connects the data points obtained with single gradients.

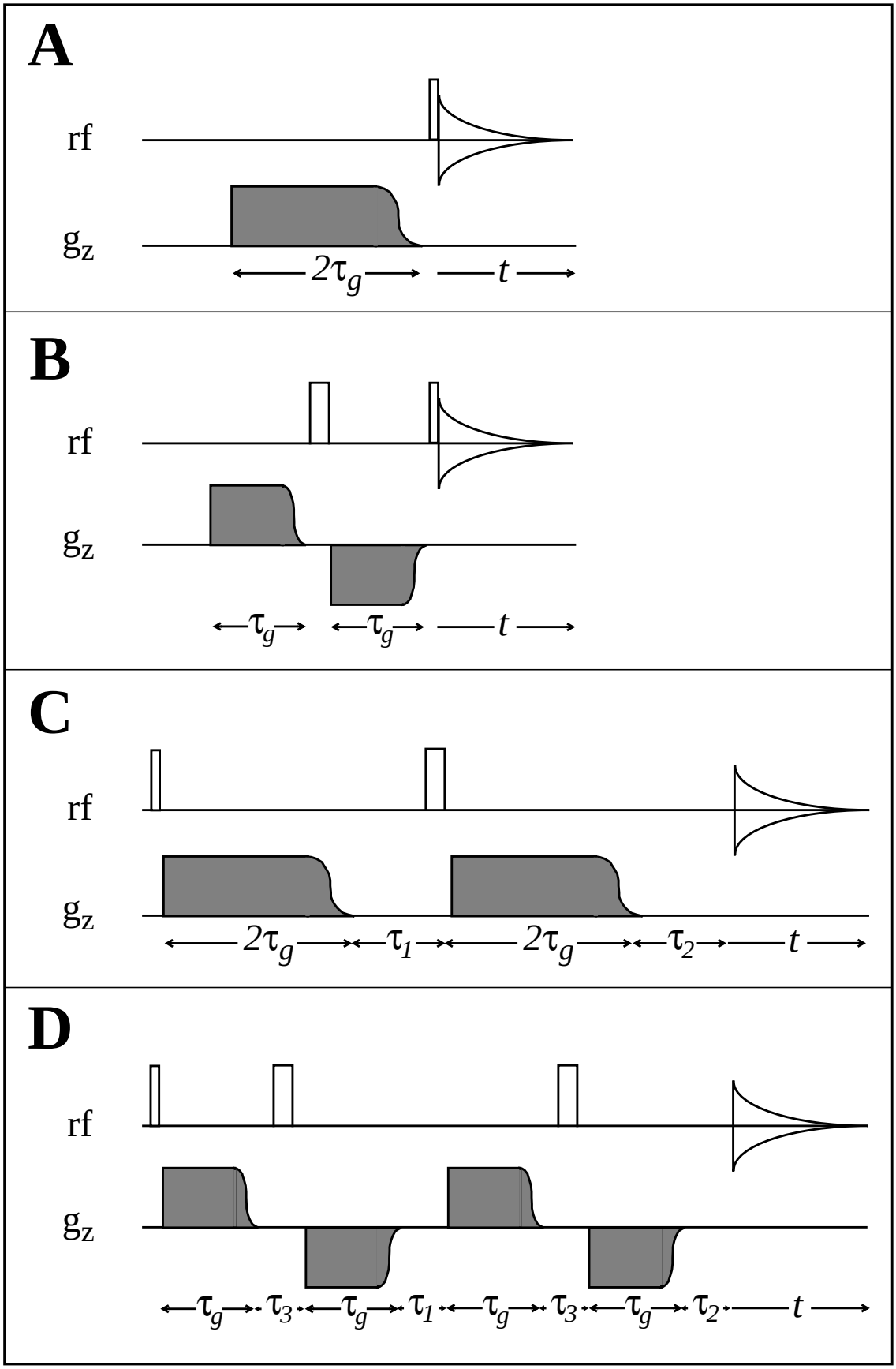
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Fig. 1

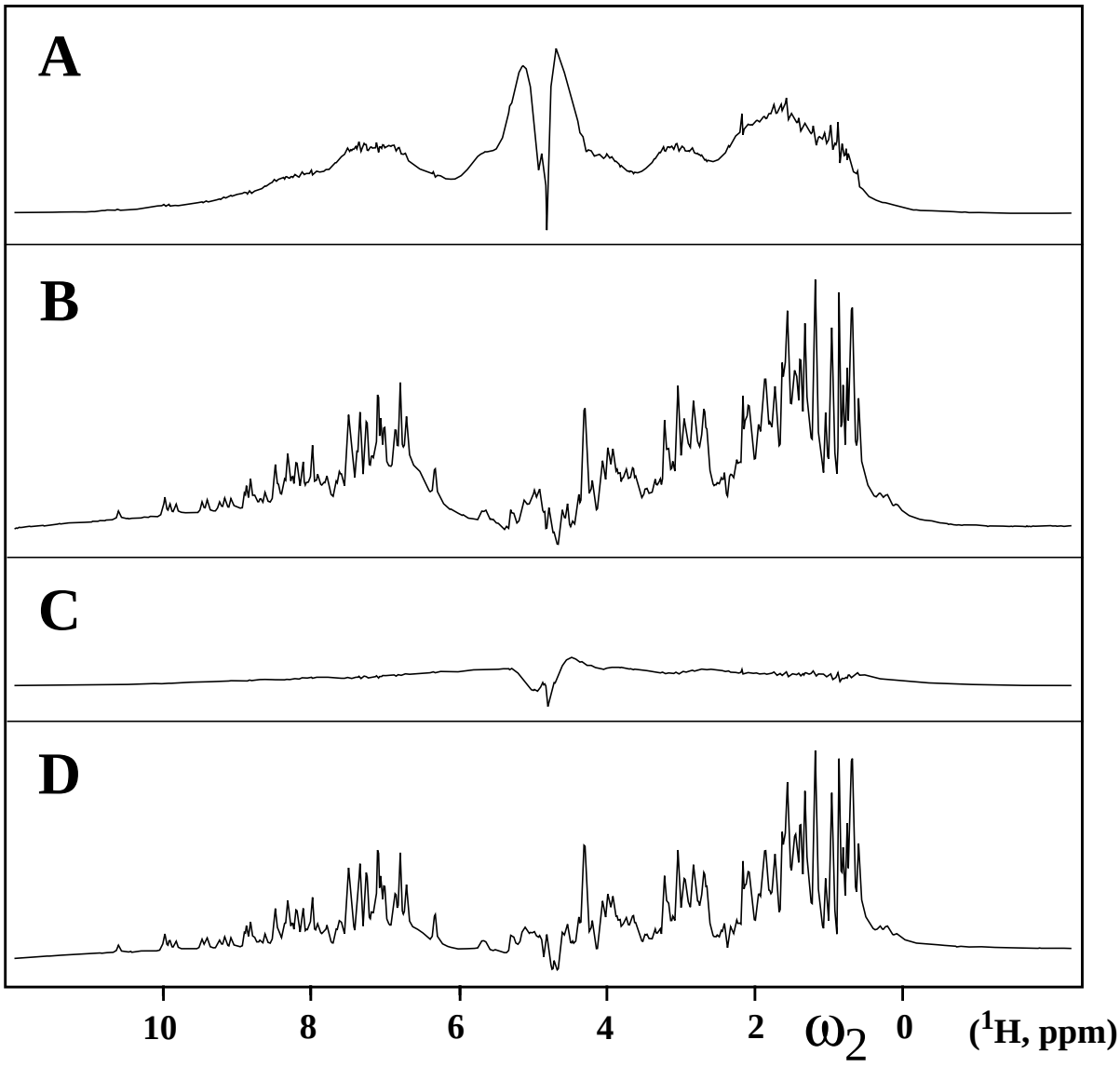

Fig. 2

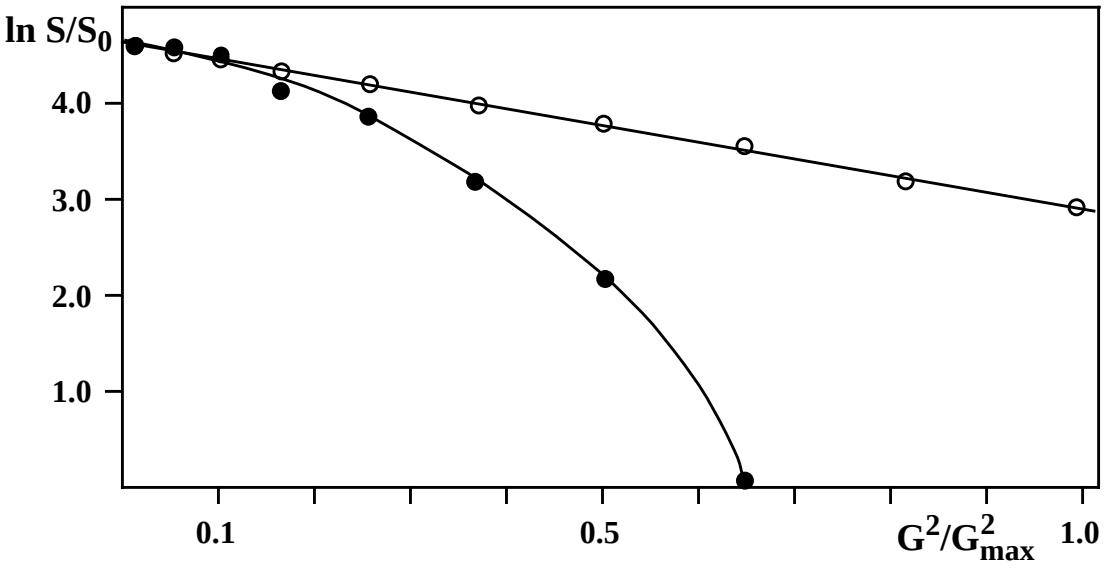

Fig. 3