Development and Applications of Advanced $(B_0$ Gradient-Based) NMR Diffusion Experiments

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STATEMENT OF AUTHENTICATION

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not previously submitted this material, either in whole or in part, for a degree at this or any other institution.
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<th>Description</th>
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<tbody>
<tr>
<td>ADC</td>
<td>Analogue-to-digital converter</td>
</tr>
<tr>
<td>Anti-SpinOrder-PGSTE</td>
<td>Anti-phase spin order stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>Asym-Anti-PGSTE</td>
<td>Asymmetric anti-phase stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>Asym-Max-PGSTE</td>
<td>Asymmetric maximum quantum stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill sequence</td>
</tr>
<tr>
<td>DIRE</td>
<td>Diffusion and relaxation editing sequence</td>
</tr>
<tr>
<td>DQ</td>
<td>Double quantum</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum (i.e., linewidth)</td>
</tr>
<tr>
<td>LSSolve</td>
<td>A Maple routine least-squares tool</td>
</tr>
<tr>
<td>MAG-PGSTE</td>
<td>Modified Cotts 17-interval sequence with gradients at ‘magic’ ratio</td>
</tr>
<tr>
<td>MAGSTE</td>
<td>Magic asymmetric gradient stimulated echo based sequence</td>
</tr>
<tr>
<td>Max-SpinOrder-PGSTE</td>
<td>Maximum quantum spin order stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>MQ</td>
<td>Multi-quantum</td>
</tr>
<tr>
<td>MSD</td>
<td>Mean-squared displacement</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PGSE</td>
<td>Pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>PGSTE</td>
<td>Stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>PGSTE-WATERGATE</td>
<td>Stimulated echo based pulsed gradient spin-echo sequence with two WATERGATE units</td>
</tr>
<tr>
<td>PM</td>
<td>Phase modulated</td>
</tr>
<tr>
<td>PM1 or PM2</td>
<td>Phase modulated binomial-like sequence</td>
</tr>
<tr>
<td>QQ</td>
<td>Quadruple quantum</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal-to-noise</td>
</tr>
<tr>
<td>STE</td>
<td>Stimulated echo</td>
</tr>
<tr>
<td>S/V</td>
<td>Surface-to-volume ratio</td>
</tr>
<tr>
<td>Sym-Anti-PGSTE</td>
<td>Symmetric anti-phase stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>Sym-Max-PGSTE</td>
<td>Symmetric maximum quantum stimulated echo based pulsed gradient spin-echo sequence</td>
</tr>
<tr>
<td>TQ</td>
<td>Triple quantum</td>
</tr>
<tr>
<td>VCF</td>
<td>Velocity correlation function</td>
</tr>
<tr>
<td>WATERGATE</td>
<td>Water suppression by gradient-tailored excitation</td>
</tr>
<tr>
<td>W3 or 3-9-19</td>
<td>A six-pulse binomial-like sequence with pulse durations in the ratio of 3:9:19:19:9:3</td>
</tr>
<tr>
<td>W5</td>
<td>A ten-pulse binomial-like sequence</td>
</tr>
</tbody>
</table>
ABSTRACT

Pulsed gradient spin-echo (PGSE) nuclear magnetic resonance (NMR) has become a method of choice for the determination of random motion (i.e., translational diffusion or self-diffusion) of molecules and small particles. This thesis focuses on the development of advanced PGSE NMR methods for conducting self-diffusion measurements on magnetically inhomogeneous (i.e., containing materials with different magnetic susceptibilities), aqueous or slowly diffusing samples.

PGSE NMR diffusion measurements rely on the accurate encoding of the position of each diffusing particle by the use of pulses of spatially well-defined magnetic gradients. However, for a magnetically inhomogeneous sample, local magnetic field gradients (i.e., background gradients) with unknown intensities and directions, which are normally generated by the differences in magnetic susceptibilities inside and/or around the sample, can also lead to the position encoding of the diffusing particles. This position encoding may couple with the position encoding caused by the applied pulsed gradients and thus cause errors in PGSE NMR diffusion measurements. Although in numerous cases background gradients are assumed to be spatially and temporally constant in order to simply the analysis of the background gradient based position encoding, in general the background gradient experienced by each diffusing particle is non-constant both spatially and temporally because of the complex sample structure and particles diffusing through different
magnetic environments. To suppress the deleterious effects of the (non-constant) background gradients, a new stimulated-echo (STE)-based PGSE method with the intensities of pulsed gradients at a magic ratio (MAG), MAG-PGSTE, was developed for the determination of self-diffusion in magnetically inhomogeneous samples. The method was tested on two water saturated glass bead packs (i.e., 212-300 µm and <106 µm glass beads). The MAG-PGSTE method was compared to the magic asymmetric gradient STE-based PGSE (MAGSTE or MPFG) method, which is newly developed for the suppression of the deleterious effects of the (non-constant) background gradients in only one transient, and Cotts 13-interval method, one of the most successful suppression methods based on the assumption of constant background gradients, using both glass bead samples. The MAG-PGSTE and MAGSTE method outperformed the Cotts 13-interval method in the measurement of diffusion coefficients; more interestingly, for the <106 µm glass bead sample with smaller bead sizes and thus higher background gradients, the MAG-PGSTE method provided higher signal-to-noise ratios and thus better diffusion measurements than the MAGSTE and Cotts 13-interval methods. In addition, when using relatively long pulsed gradients (e.g., 3 ms), the MAG-PGSTE method provided good bead size characterizations.

Due to solubility problems, limited sample availability and/or aggregation, solvent (e.g., water) signals in NMR are typically 4-5 orders of magnitude higher than the solute signals. The huge solvent signal not only overlaps with the signals of interest but also saturates the NMR receiver and thus prevents the detection of the weak signals from the molecules of interest. To achieve a high degree of water suppression in PGSE NMR diffusion measurements, a new STE-based PGSE method incorporating the WATERGATE method, which is based on the selective manipulation of the water signal by the use of a binomial-like selective inversion
sequence (i.e., a group of radio frequency (RF) pulses with symmetric pulse duration arrangement separated by equal delays), PGSTE-WATERGATE, was developed. The method is simple to set up and particularly suited to measuring diffusion coefficients in aqueous solution, which is commonly required in pharmaceutical and combinatorial applications. The method was tested on a sample containing lysozyme in 10% $^2\text{H}_2\text{O}$ and 90% $^1\text{H}_2\text{O}$ and a sample containing sucrose in 10% $^2\text{H}_2\text{O}$ and 90% $^1\text{H}_2\text{O}$ and provided superb water suppression and accurate diffusion measurements. Importantly, the new method provided the high degree suppression of the phase distortions in NMR spectra caused by the use of selective inversion pulses or sequences.

In the NMR spectrum of an aqueous (bio-molecular) sample, especially a protein sample, the water signal almost always overlaps with the signals of interest. Therefore, a good water suppression technique should target only the water signal. The selectivity of the PGSTE-WATERGATE method depends on the selectivity of the binomial-like selective inversion sequence. The traditional way to enhance the selectivity of a binomial-like sequence is to increase the number of RF pulses contained in the binomial-like sequence. This type of selectivity enhancement is very effective but leads to longer sequence durations and thus the signal loss due to NMR relaxation. In this research, two 6-pulse phase-modulated binomial-like inversion sequences were developed by simultaneously optimizing the RF pulse durations and phases instead of increasing the number of the RF pulses. In combination with the excitation sculpting method, which contains two WATERGATE unit and affords the suppression of phase distortions caused by the phase modulations on the binomial-like sequences, both of the new binomial-like sequences outperformed the well-known 3-9-19 sequence, a 6-pulse binomial-like sequence with a pulse duration ratio of 3:9:19:19:9:3, in selectivity and inversion width. The new sequences provided the
similar selectivity and inversion width to the W5 sequence, a 10-pulse binomial-like sequence, but with significantly shorter sequence durations. When used in PGSTE-WATERGATE, they afforded highly selective water suppression in diffusion experiments.

The hydrodynamics of slowly diffusing molecules (e.g., protein) and small particles (e.g., protein aggregates) has long been of scientific interest due to its importance in the understanding of many biological and biophysical processes such as protein crystallization. However, the diffusion measurements on these molecules and small particles confront the PGSE NMR methods with huge challenges because the diffusion coefficients of these molecules and small particles are normally at or under the lower diffusion determination limit of the PGSE NMR methods, which is mainly controlled by the maximum pulsed gradient strength of the NMR probe (i.e., high pulsed gradient strengths are preferred for measuring slow diffusion), the pulsed gradient durations (i.e., long gradient durations are preferred for measuring slow diffusion), and the observation time (i.e., long observation times are preferred for measuring slow diffusion) which is limited by the NMR relaxation times of the molecules and small particles. To overcome this problem, multi-quantum NMR coherences may be utilized in PGSE NMR experiments as these coherences are more sensitive to the encoding by the pulsed gradients than normal single quantum coherences (e.g., the encoding effect of a pulsed gradient on a single quantum coherence may be doubled by using a double-quantum coherence). In this research, five new multi-quantum coherence encoding STE-based PGSE methods were successfully developed. The new multi-quantum PGSE methods were tested on a sample containing L-[3-13C]-alanine in 2H2O. The quadruple quantum and triple
quantum coherences from $^{13}\text{CH}_3$, which can greatly enhance the encoding by pulsed gradients, were successfully selected using these new sequences.

This thesis contains eight chapters. In Chapter 1, the focus of this thesis, translational diffusion, is introduced and the difficulties in the PGSE NMR diffusion measurements on magnetically inhomogeneous, aqueous, and slowly diffusing samples are also introduced. In Chapter 2, to facilitate the understanding of how PGSE NMR diffusion measurements work, both the basic principles and simulation methods of NMR and the fundamentals of PGSE NMR diffusion measurements are given. In Chapter 3, a brief review on the current advanced PGSE NMR methods for measuring diffusion in magnetically inhomogeneous, aqueous, and slowly diffusing samples is given. In Chapter 4, the experimental details of the research work in this thesis are given. From Chapter 5 to Chapter 7, newly developed PGSE NMR methods and their applications are discussed in detail. In Chapter 8, the general conclusions are given.
Chapter 1    Introduction

1.1 Translational Diffusion

1.1.1 Isotropic and Anisotropic Translational Diffusion

Translational diffusion (also referred to as self-diffusion, Brownian motion, or random walks) is the random movement of molecules or small particles resulting from thermal energy [1-4]. For an isotropic system (e.g., infinitely diluted solution) the self-diffusion can be characterized by the isotropic self-diffusion coefficient \( D, \text{ m}^2 \text{ s}^{-1} \) while for an anisotropic system (e.g., human tissue) the self-diffusion should be characterized by the diffusion tensor [5], \( \mathbf{D} \), which can be written as

\[
\mathbf{D} = \begin{bmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{bmatrix}.
\]  (1.1)

The diagonal elements of \( \mathbf{D} \) scale the self-diffusion processes at the same direction and the off-diagonal elements correlate the self-diffusion processes in orthogonal directions. Henceforth, however, we are only concerned with isotropic self-diffusion.

1.1.2 Stokes–Einstein–Sutherland Equation

Self-diffusion of molecules and small particles is affected by many factors including
temperature, molecular weight, and viscosity. Using the Stokes-Einstein (also referred to as Stokes–Einsteiń–Sutherland) equation [6-8], the diffusion coefficient can be related to the factors mentioned above by [6-8]

\[ D = \frac{kT}{f}, \]  

(1.2)

where \( k \) is the Boltzmann constant, \( T \) is temperature, and \( f \) is the friction coefficient. Although the friction coefficient takes different forms depending on the shape of the diffusing particle [9], for a spherical molecule with a Stokes radius (i.e., effective hydrodynamic radius) \( r_s \), which normally increases with the increase of molecular weight (\( M_w \)), in a medium of viscosity \( \eta \), the friction factor is given by

\[ f = 6\pi r_s \eta. \]  

(1.3)

Neglecting the influences of molecular shapes, Eq. (1.2) simply indicates that \( D \) decreases when \( M_w \) increases. This trend is shown in Table 1-1. Due to the strong correlation between \( D \) and \( M_w \), in chemi-physical and biophysical systems \( D \) carries molecular size information and thus thermodynamic information about chemi-physical and biophysical processes such as protein association.
Table 1-1 Diffusion coefficients (at 25 ºC) of some molecules with different M_w’s.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Diffusion Coefficient (10^{-9} \times m^2 s^{-1})</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_2O</td>
<td>2.3a</td>
<td>18</td>
</tr>
<tr>
<td>Residual ^1H^2HO in ^2H_2O</td>
<td>1.9b</td>
<td>19</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.4b</td>
<td>32</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>1.4b</td>
<td>84</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>4.2b</td>
<td>86</td>
</tr>
<tr>
<td>Cyclooctane</td>
<td>0.6b</td>
<td>112</td>
</tr>
<tr>
<td>2 mM lysozyme in 10% ^2H_2O and 90% H_2O</td>
<td>0.1c</td>
<td>&gt; 10000</td>
</tr>
</tbody>
</table>

a. From reference [10].
c. From reference [12].

1.1.3 Diffusion Propagators

In most cases, self-diffusion should be strictly distinct from mutual diffusion, which is driven by concentration gradients and characterized by a mutual diffusion coefficient (D_M, m^2 s^{-1}). However, for a homogeneous single-component system or infinitely dilute solution the (single) mutual diffusion coefficient equals the self-diffusion coefficient [1, 13]; therefore, Fick’s laws, which describe mutual diffusions, provide the foundation of the mathematical description of self-diffusion for these systems. For a homogeneous single-component system or an infinitely dilute solution, the concentration change over time can be related to the mutual diffusion by the use of Fick’s second law of diffusion (e.g., [4])

\[
\frac{\partial c(r,t)}{\partial t} = D_M \nabla^2 c(r,t),
\]  (1.4)
where \( c(\mathbf{r}, t) \) is the particle concentration at position \( \mathbf{r} \) at time \( t \). For a self-diffusion process in the absence of any concentration gradients, the total probability \( (P(\mathbf{r}_1, t)) \) of finding a particle at position \( \mathbf{r}_1 \) at time \( t \) is given by (e.g., [14])

\[
P(\mathbf{r}_1, t) = \int \rho(\mathbf{r}_0)P(\mathbf{r}_0, \mathbf{r}_1, t) \, d\mathbf{r}_0,
\]

(1.5)

where \( \rho(\mathbf{r}_0) \) is the particle density at position \( \mathbf{r}_0 \), which is given by

\[
\rho(\mathbf{r}_0) = \lim_{t \to 0} P(\mathbf{r}_0, \mathbf{r}_1, t) \, d\mathbf{r}_1,
\]

(1.6)

and \( P(\mathbf{r}_0, \mathbf{r}_1, t) \) is the conditional probability of finding a particle initially at position \( \mathbf{r}_0 \), at position \( \mathbf{r}_1 \) after a time \( t \). Because \( P(\mathbf{r}_1, t) \) is based on an ensemble average, it follows Fick’s second law (e.g., [15]). Therefore, noting \( D = D_M \) (NB, for homogeneous single-component systems or infinitely dilute solutions) substituting \( P(\mathbf{r}_0, \mathbf{r}_1, t) \) for \( c(\mathbf{r}, t) \) in Eq. (1.4) gives

\[
\frac{\partial P(\mathbf{r}_0, \mathbf{r}_1, t)}{\partial t} = D \nabla^2 P(\mathbf{r}_0, \mathbf{r}_1, t)
\]

(1.7)

with the initial condition,

\[
P(\mathbf{r}_0, \mathbf{r}_1, 0) = \delta(\mathbf{r}_1 - \mathbf{r}_0),
\]

(1.8)

here \( \delta \) is the Dirac delta function.

\( P(\mathbf{r}_0, \mathbf{r}_1, t) \) is known as Green’s function or diffusion propagator [16, 17]. \( P(\mathbf{r}_0, \mathbf{r}_1, t) \) may take different forms for different boundary conditions (i.e., the geometry of
the medium in which the particles diffuse) [14, 18]. For the boundary condition of $P \rightarrow 0$ as $r_1 \rightarrow \infty$ (i.e., self-diffusion in a homogeneous medium), $P(r_0, r_1, t)$ can be obtained from Eq. (1.8) as a Gaussian function [18]

$$P(r_0, r_1, t) = (4\pi Dt)^{3/2} \exp\left(-\frac{(r_1 - r_0)^2}{4Dt}\right).$$

As shown in Eq. (1.9), $P(r_0, r_1, t)$ depends only on the dynamic displacement, $r_1 - r_0$ (NB for the self-diffusion in homogeneous medium).

In following Sections and Chapters, the terms ‘diffusion’ and ‘self-diffusion’ are synonymous.

### 1.1.4 Mathematical Description of Diffusion

Related to the research work in this thesis are two descriptions of diffusion: the mean-squared displacement (MSD)-based description and the velocity correlation function (VCF)-based description.

The MSD of a diffusing particle is given by (e.g., [17, 19])

$$\langle (r_1 - r_0)^2 \rangle = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} (r_1 - r_0)^2 \rho(r_0) P(r_0, r_1, t) dr_0 dr_1,$$  \hspace{1cm} (1.10)

where $\langle \rangle$ denotes an average over an ensemble of particles. For a Gaussian form diffusion propagator, the mean–squared displacement can be related to the diffusion coefficient by (e.g., [20])

$$\langle (r_1 - r_0)^2 \rangle = nDt,$$  \hspace{1cm} (1.11)
where \( t \) (s) is time and \( n = 2, 4, \) or \( 6 \) for one, two, or three dimensions, respectively.

The VCF of a diffusing particle is given by [15]

\[
\langle v_\alpha (0) v_\alpha (t) \rangle = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \rho (r_0) P (r_0, r_1, t) v_\alpha (r_0) v_\alpha (r_1) \, dr_0 \, dr_1 ,
\]

where \( v_\alpha (0) \) is the initial velocity of the particle along the direction ‘\( \alpha \)’ (\( \alpha = x, y, \) or \( z \)), \( v_\alpha (t) \) is the velocity at time \( t \), \( v_\alpha (r_0) \) is the velocity of the particle at position \( r_0 \), and \( v_\alpha (r_1) \) is the velocity of the particle at position \( r_1 \). The Fourier transform of the velocity correlation function is the diffusion spectrum [15]

\[
D(\omega) = \int_0^\infty \langle v_\alpha (0) v_\alpha (t) \rangle \exp (i \omega t) \, dt ,
\]

where \( \omega \) is the frequency of the velocity correlation function.

Both the diffusion coefficient \( D \) and the diffusion spectrum \( D(\omega) \) can be used to describe diffusion. \( D \) and \( D(\omega) \) can be related as follows:

\[
\left\langle (r_i - r_0)^2 \right\rangle \text{ can be evaluated from } \left\langle v_\alpha (t') v_\alpha (t^*) \right\rangle \text{ by [21]}
\]

\[
\left\langle (r_i - r_0)^2 \right\rangle = \int_0^t \int_0^t \left\langle v_\alpha (t') v_\alpha (t^*) \right\rangle \, dt' \, dt^* .
\]

Combining Eq. (1.13) and Eq. (1.14) gives [22]
Combining Eq. (1.11) and Eq. (1.15) gives the desired relation

\[
\langle (r_i - r_0)^3 \rangle = \frac{1}{\pi} \int_{-\infty}^{\infty} D(\omega) \frac{\sin^2(\omega t/2)}{(\omega/2)^3} d\omega.
\]

(1.15)

\[
D = 2\pi t \int_{-\infty}^{\infty} D(\omega) \frac{\sin^2(\omega t/2)}{(\omega/2)^3} d\omega.
\]

(1.16)

### 1.1.5 Free and Restricted Diffusion

In practice, especially in the liquid phase, diffusing species are always restricted by certain boundaries (e.g., water in a small tube and water inside glass bead packs). As shown in Figure 1–1, diffusing molecules inside a spherical boundary can be divided into two groups: the molecules inside the bulk (i.e., far away from the boundary) and the molecules close to the boundary. At relatively short observation or diffusion times (e.g., 1 ms for water molecules diffusing inside a spherical boundary with a diameter of 200 μm at room temperature) the molecules in the bulk don’t feel the boundary at all (i.e., free diffusion) but the molecules close to the boundary feel the restrictions (i.e., restricted diffusion) [23, 24]; while, at relatively long observation times (e.g., 10 s for water molecules diffusing inside a spherical boundary with a diameter of 10 μm at room temperature) all the molecules can diffuse a longer distance and thus start to feel the restrictions. Therefore, at short diffusion times both free and restricted diffusion exist but free diffusion dominates while at long diffusion times restricted diffusion dominates.

Although Eq. (1.9) and (1.11) are strictly not valid for restricted diffusion, these two equations can still be used, especially at short observation times (Figure 1–1) [24, 25], for diffusion data analysis and leads to the apparent diffusion coefficient
(also termed as the measured diffusion coefficient or time-dependent diffusion coefficient (e.g., [25])), which conveys structural information of the boundaries. For detailed understanding of restricted diffusion, please read specialized books and review papers (e.g., [2, 4, 14, 17, 20, 24, 26]).

Figure 1–1 Molecules diffusing inside a boundary at short and long observation times. The interactions between diffusing particles and the boundary are highly simplified into ‘bounces’; however, in practice these interactions are far more complicated.

1.1.6 Diffusion Measurements

Although there are numerous methods for measuring diffusion such as light scattering [27], fluorescence [28], capillary [29] and nuclear magnetic resonance (NMR) [30], NMR based methods outperform the others due to their non-invasive nature (i.e., NMR based methods don’t affect the thermodynamics of a system). In general, NMR based diffusion measurements can be classified into relaxation based methods (e.g., [31]) and pulsed field gradient spin-echo (PGSE) methods (e.g., [13]). The relaxation based methods measure the molecular rotation occurring in the picosecond to nanosecond range while the PGSE methods measure translational motion occurring on the timescale of millisecond to second (e.g., [20]).
For the relaxation based methods, the rotational correlation time ($\tau_c$) [32] of the studied particles, which is obtained from analysis of NMR relaxation data, is related to the medium viscosity and then to the diffusion coefficient by the use of the Debye equation [33, 34]

$$\tau_c = \frac{4\pi\eta r^3}{3kT}$$

(1.17)

and the Stokes–Einstein–Sutherland equation (Eq. (1.2)). The validity of the relaxation based methods is based on a number of assumptions: firstly, the relaxation mechanism of the diffusing particles must be known; secondly, owing to the strong dependence of these methods on the Debye and Stokes–Einstein–Sutherland equations, the diffusing particles must see a continuous bulk medium [31] and the Stokes radius of the diffusing particles must be known. Therefore, the application of the relaxation based methods is highly limited.

In contrast, the PGSE methods work without any assumptions. However, analysis of PGSE data requires the diffusion propagator be known. A PGSE experiment generally includes three steps: firstly, a pulsed magnetic field gradient (group) is applied to the sample to encode the position of each diffusing particle; secondly, a delay (i.e., observation time or diffusion time) is given for observing the diffusion of the encoded particles; finally, at the end of the delay, another identical pulsed magnetic field gradient (group) is applied so that the position of each particle can be decoded (i.e., inversely encoded). Without diffusion, a complete decoding can be observed while diffusion leads to an incomplete decoding and thus an attenuation of the observed NMR signal. The value of the signal attenuation is directly related to the value of the diffusion coefficient or apparent diffusion coefficient and thus can be
used for diffusion measurements. Detailed discussions of the PGSE methods can be found in Chapter 2.

1.2 Magnetic Flux Density and Magnetic Susceptibility

In a medium, the magnetic flux density $B$ (T), which is usually referred to as the magnetic field in NMR experiments, established by an applied magnetic field $H$ (A m$^{-1}$) can be calculated by [35, 36]

$$B = \mu_0 (1 + \chi) H ,$$  \hspace{1cm} (1.18)

where $\mu_0$ is the magnetic permeability of free space ($4\pi \times 10^{-7}$ H m$^{-1}$) and $\chi$ (dimensionless) is the magnetic susceptibility which expresses how readily the medium develops a magnetic moment on exposure to an external magnetic field. $\chi$ takes zero for vacuum and for materials it may take positive (for paramagnetic materials) or negative (for diamagnetic materials) values. In all following discussions, $B$ is referred to as the magnetic field. A paramagnetic sample tries to pull the magnetic field into the material and thus causes a stronger magnetic field than the applied magnetic field while a diamagnetic sample tries to push the magnetic field out of the material and thus leads to a weaker magnetic field.

From Eq. (1.18), it can be realized that the differences in the magnetic susceptibility creates spatial magnetic field variations (background gradients) in the sample [37]. As shown in Figure 1–2, background gradients are generated around a diamagnetic object in vacuum due to the difference in the magnetic susceptibility. In some cases, the amplitude of the background gradients can be of the order of T m$^{-1}$, which is similar to the strength of applied magnetic field gradients in PGSE diffusion.
experiments. For example, it is estimated that red blood cells have gradients up to $2 \times 10^{-2} \text{T m}^{-1}$ due to the large difference in the magnetic susceptibility between the inside and outside of the cells [38, 39]; in metal hydride samples, such background gradients can be of the order of $0.5 \text{T m}^{-1}$ because of differences in magnetic susceptibility between different materials [40]. These samples, containing significant susceptibility inhomogeneity, are referred to as magnetically inhomogeneous samples. Even in a glass tube containing a homogeneous sample such as water, background gradients occur due to the difference in magnetic susceptibility between the water and the air that surrounds the sample and tube [41].

![Conceptual diagrams for the magnetic field distorted by an object with a negative magnetic susceptibility ($\chi < 0$) in vacuum. The distortion has been greatly exaggerated for the sake of clarity.](image)

**1.3 Difficulties in PGSE Diffusion Measurements on Magnetically Inhomogeneous, Aqueous and Slowly Diffusing Samples**

(Only a brief introduction is given here; for detailed discussions please refer to Chapter 3.)

PGSE NMR diffusion measurements rely on the accurate encoding of the position of
each diffusing particle by the use of pulsed magnetic field gradients. However, for a magnetically inhomogeneous sample, local magnetic field gradients with unknown intensities and directions, which are generated by the differences in magnetic susceptibility inside and/or around the sample, can also lead to a position encoding process. This position encoding may couple with that caused by the applied pulsed gradients and thus cause errors in PGSE NMR diffusion measurements.

Due to solubility problems, limited sample availability and/or aggregation, the water signal in NMR is typically 4-5 orders of magnitude higher than the solute signals. The huge water signal not only overlaps with signals of interest but also saturates the NMR signal receiver and thus prevents the detection of the weak signals from molecules of interest.

The hydrodynamics of slowly diffusing molecules (e.g., protein) and small particles (e.g., protein aggregates) has long been of scientific interest due to its importance in the understanding of many biological and biophysical processes such as protein crystallization. However, the diffusion measurements on these molecules and small particles confront the PGSE NMR methods with huge challenges because the diffusion coefficients of these molecules and small particles are normally at or under the lower diffusion determination limit of the PGSE NMR method.

1.4 Aims

The aims of the research in this thesis were: firstly, to develop new advanced PGSE NMR diffusion experiments which can afford accurate diffusion measurements on magnetically inhomogeneous, aqueous, and slowly diffusing samples; secondly, to gain accurate sphere size characterization, precise diffusion determination on aqueous samples, and good multi-quantum (MQ) coherence selectivity by using these new methods, respectively. By performing this research, contributions have been made to
NMR-based micro-structure characterization, bio-molecular NMR, and hydrodynamic studies on macromolecules.
Chapter 2  Theory of NMR methods

Some concepts of NMR and PGSE, essential to the research work in this thesis, are reviewed here. To gain further understanding of these concepts, please read the specialized books and review papers (e.g., [32, 42] for NMR and [13, 15, 20, 43-46] for PGSE). A brief introduction of product operator formalism [47] is also given here due to the intensive use of product operators in this work.

2.1 Basic NMR Theory

2.1.1 Nuclear Spins, Nuclear Magnetization, Spin Precession and Resonance

Every nucleus has an intrinsic but extremely intangible (i.e., compared with mass and charge) property called “spin”, which gives the nucleus an intrinsic angular momentum. Although the spin angular momentum is extremely intangible, it is closely related to another intrinsic property of nuclei, the nuclear magnetic moment. The nuclear spin angular momentum is either parallel (i.e., for nuclei with positive gyromagnetic ratios ($\gamma$, rad s$^{-1}$ T$^{-1}$)) or in the opposite direction (i.e., for nuclei with negative $\gamma$ values) to the nuclear magnetic moment. It is the nuclear magnetic moment that makes the spin visible in NMR spectrometers. The spin is characterized by a quantum number, $I$; the guidelines for the evaluation of the value of $I$ are: $I = \text{half integer}$ for nuclei with odd mass numbers (e.g., $I = 1/2$ for $^{13}$C and $^1$H), $I = \text{a non-zero}$
integer for nuclei with odd proton numbers and odd neutron numbers (e.g., \( I = 1 \) for \(^2\text{H}\)), and \( I = 0 \) for nuclei with even proton numbers and even neutron numbers (e.g., \( I = 0 \) for \(^{12}\text{C}\)) (e.g., [32]). Because only spins with \( I = 1/2 \) (i.e., spin-1/2) were studied in the research work in this thesis, all the discussions in this Chapter are restricted to spin-1/2. When the spins are put into an applied magnetic field (\( \mathbf{B}_0 \), a vector quantity with a direction arbitrarily assigned along the \( z \)-axis), the spins will take different energy levels (i.e., \((2I + 1)\) eigenstate levels or their superpositions [32]) due to the interactions between the nuclear magnetic moments and \( \mathbf{B}_0 \).

![Conceptual diagrams](image_url)

Figure 2–1 Conceptual diagrams for the distribution of spins in the (A) absence and (B) presence of an applied magnetic field. The anisotropy caused by the applied magnetic field has been greatly overstated for the sake of clarity.

After the spins stay inside the applied magnetic field for a certain period of time, a stable net anisotropic distribution of the spin polarizations (i.e., thermal equilibrium) will be reached [32], the vector sum of the spins will slightly deviate from zero (i.e., longitudinal relaxation, see Section 2.1.2; Figure 2–1), and thus a weak net magnetization (\( \mathbf{M} \), \( \text{A m}^{-1} \)) is generated. The net magnetization precesses around \( \mathbf{B}_0 \) like a tilted spinning top when it deviates from the direction of \( \mathbf{B}_0 \). The
Angular velocity of the precession can be calculated from the Larmor equation (e.g., [32]):

\[
\omega = -\gamma B_0,
\]

(2.1)

where \(\omega\) is the Larmor frequency (rad s\(^{-1}\)) and \(B_0\) is the strength of the magnetic field (T).

Figure 2–2 The nutation effect of a 90° RF pulse with a phase of \(x\), and the precession of the nutated bulk nuclear magnetization around \(B_0\). The precessing magnetization induces currents in the receiver coil. To achieve quadrature detection, the NMR signal is split into two paths and then these two signals are multiplied by two synthesized RF signals which have a phase difference of \(\pi/2\), respectively (e.g., [32]).

If another weak magnetic field, \(B_1\), which is generated by pulsed radio frequency (RF) electromagnetic radiation (i.e., an RF pulse), is applied in a particular direction perpendicular to \(B_0\), it will rotate \(M\) and thus change the angle between \(M\) and \(B_0\) (i.e., the nutation angle). When the RF pulse has a frequency the same as the Larmor frequency of the spins of interest (i.e., on-resonance), it will rotate \(M\) purely
around $B_1$. For an on-resonance RF pulse of duration $t_p$, the nutation angle, $\theta$, is given by:

$$\theta = \gamma B_1 t_p.$$  \hfill (2.2)

Two important properties of an RF pulse are the nutation angle ($\theta$) and the pulse phase (i.e., the direction of the nutation axis). For example, a 90° RF pulse with a phase of $x$ (i.e., denoted 90°, 90°($x$), $\pi/2$, or $\pi/2(x)$) rotates $M$ 90° around the $x$ axis (Figure 2–2).

### 2.1.2 Relaxation, Bloch equations, FIDs, and Fourier Transforms

In NMR, relaxation simply means the loss of energy and/or phase coherence. Two types of relaxations affect NMR experiments: spin-lattice (longitudinal) relaxation and spin-spin (transverse) relaxation. If $M$ has been nutated away from the direction of $B_0$ by the application of RF pulses, it tends to re-align along $B_0$ by longitudinal relaxation (i.e., a process in which the aligned spins lose energy to the environment until the thermal equilibrium is reached) in an approximately exponential process characterized by the rate $1/T_1$ (Figure 2–3A); $T_1$ is the longitudinal relaxation time constant. The term “lattice” comes from solid-NMR, where the longitudinal relaxation is controlled by the interactions between the spins and crystal lattice [32]. During transverse relaxation, the aligned spins lose phase coherence by the rate of $1/T_2$ (Figure 2–3B); $T_2$ is the transverse relaxation time constant. This specific dephasing is mainly due to the interactions between the nuclear magnetic moments and the fluctuating microscopic magnetic fields caused by the random movement of spin-containing molecules (i.e., primarily rotational diffusion and to a lesser extent
translational diffusion) (e.g., [32]).

A

Figure 2–3 (A) Schematic diagram of longitudinal relaxation. After being nutated away from the $z$-axis (i.e., direction of the static $B_0$ field) by an RF pulse at the resonance frequency (i.e., Larmor frequency), the bulk magnetization starts to return to thermal equilibrium value via longitudinal relaxation. (B) Schematic diagram of transverse relaxation. After being nutated into the $x$-$y$ plane, the bulk magnetization starts to dephase due to fluctuating microscopic magnetic fields. To illustrate the two relaxation processes more clearly, the longitudinal and transverse relaxation are shown separately although both processes always occur at the same time.

Neglecting diffusion and chemical exchange, the evolution of $M$ in the presence of longitudinal and transverse relaxation can be described by the Bloch equations [48]:

$$ \frac{\partial M(r, t)}{\partial t} = \gamma M \times B(r, t) - \frac{M_x i + M_y j}{T_2} - \frac{(M_z - M_0) k}{T_1}, \quad (2.3) $$

where $r$ is position, $M_x$, $M_y$, and $M_z$ are the magnitudes of $M$’s $x$, $y$, and $z$ component, respectively, $i$, $j$, and $k$ are Cartesian unit vectors, $M = M_x i + M_y j + M_z k$, $M_0$ is the
bulk magnetization at equilibrium, and $T_2^*$ includes the effect of transverse relaxation and macroscopic magnetic inhomogeneity (i.e., background gradients).

Due to the effects of transverse relaxation and macroscopic magnetic inhomogeneity, the precessing $\mathbf{M}$ generates a decaying sinusoidal current (i.e., the ‘raw’ NMR signal) oscillating at a very high frequency (e.g., a few hundred MHz for protons inside a 10 T magnetic field). The ‘raw’ analogue NMR signal has to be transformed into digital signal before being processed by the computer. However, the fast oscillating ‘raw’ NMR signal is far above the frequency limit of current analogue-to-digital converters (ADCs). Therefore, in practice, the ‘raw’ NMR signal is ‘down-converted’ by comparing it with a reference wave of frequency $\omega_{\text{ref}}$ by the use of quadrature receiver to generate a new signal oscillating at the relative Larmor frequency: (e.g., [32])

$$\Omega^0 = \omega^0 - \omega_{\text{ref}}. \tag{2.4}$$

The quadrature receiver provides two output signals of the form (e.g., [32])

$$s_A(t) \sim \cos(\Omega^0 t) \exp(-\lambda t), \tag{2.5}$$

and

$$s_B(t) \sim \sin(\Omega^0 t) \exp(-\lambda t). \tag{2.6}$$

The attenuation factor $\exp(-\lambda t)$ can be decomposed into

$$\exp\left(-\frac{t}{T_{\text{inhomo}}}\right) \exp\left(-\frac{t}{T_2}\right), \tag{2.7}$$
where \( \exp(-t/T_{\text{inhomo}}) \) describes the signal attenuation due to \( B_0 \) inhomogeneity and/or differences in magnetic susceptibility. \( s_A(t) \) and \( s_B(t) \) form a single complex signal \( s(t) \) (e.g., [32]):

\[
s(t) = s_A(t) + is_B(t) \sim \exp\left\{\left[i\Omega^0 - \lambda\right] t\right\}. \tag{2.8}
\]

Because this complex NMR signal contains both sine and cosine terms, it can not only tell us the amplitude of the Larmor frequency relative to the reference wave but also the sign of \( \Omega^0 \). A plot of \( s(t) \) versus time is known as a free induction decay (FID).

Nuclei in different electronic environments generated by different chemical structures have different Larmor frequency values (i.e., ‘chemical shifts’); therefore, the FID of a sample containing nuclei in different electronic environments is a sum of \( s(t) \)’s with different frequencies. The frequency information (i.e., chemical structure information) contained in the FID (i.e., the sum of \( s(t) \)’s) can be disclosed by Fourier transformation of the signal (e.g., [49, 50]). The Fourier transform of the FID gives the absorption mode NMR signal, which can be described by (e.g., [32])

\[
S(\omega) = \sum_l a_l \frac{\lambda}{\lambda^2 + (\omega - \omega_l^0)}, \tag{2.9}
\]

where \( \omega_l^0 \) is the resonance frequency of nuclei \( l \), \( a_l \) is the signal amplitude of nuclei \( l \), and \( \lambda = 1/T_{\text{inhomo}} + 1/T_2 \).

### 2.1.3 Magnetic Gradient

Just like other types of gradients (e.g., concentration gradients), the magnetic field
gradient, $g$ (T m$^{-1}$), simply means the position dependence of the magnetic field. There are generally two types of magnetic field gradients: the applied magnetic gradients generated by gradient coils and the background gradients generated by magnetic susceptibility inhomogeneities. The former are used for PGSE diffusion measurements while the latter cause errors in PGSE diffusion measurements. If a magnetic field gradient is applied in the presence of $B_0$, $\omega$ becomes spatially dependent (e.g., [32]),

$$\omega_{\text{eff}}(r) = \gamma_{\text{eff}} (B_0 + g \cdot r)$$

(2.10)

where $\gamma_{\text{eff}}$ is the sum of the gyromagnetic ratios involved in the coherence, for example, $\gamma_{\text{eff}} = 2\gamma_I + \gamma_S$ for the triple quantum coherence (TQ) of $4I_x I_y S_z$, and $g$ is the gradient of the magnetic field component parallel to $B_0$, i.e., (e.g., [32])

$$g = \nabla B_z = \frac{\partial B_z}{\partial x} i + \frac{\partial B_z}{\partial y} j + \frac{\partial B_z}{\partial z} k.$$  

(2.11)

As indicated by Eq. (2.10), the Larmor frequency becomes directly related to the position of the spin with respect to the direction of the gradient and thus can be used as a spatial label in the presence of a homogeneous gradient of known amplitude.

As shown in Figure 2–4, the application of a magnetic field gradient generates a magnetization vector helix and thus removes all net transverse magnetization. The helix can be fully unwound by the application of a magnetic gradient along the opposite direction with the same strength and duration as the previously applied magnetic gradient.
Figure 2–4 The magnetization vector helix with a pitch of $2\pi/(\gamma g \delta)$ (m) is generated by a magnetic field gradient along the $z$-direction (i.e., the direction of $B_0$) with a strength of $g$ and a duration of $\delta$. A stronger and/or longer gradient pulse would generate a tighter helix (i.e., a helix with a finer pitch), and spins with higher $\gamma$ values (e.g., $\gamma_{1H} > \gamma_{13C}$ and $\gamma_{\text{multi-quantum}} > \gamma_{\text{single-quantum}}$) also forms a tighter helix. The helix can be completely unwound by the application of a magnetic gradient pulse of opposite polarity (i.e., along the opposite direction) but the same strength and duration as the previous gradient.

2.1.4 Product Operators and Coherence Level

Definitions and nomenclature of product operators

Density operators or density matrices can elegantly describe the quantum state of a spin ensemble (e.g., [32]). However, using density operators is sometimes tedious for the description of the effects of pulse sequences on weakly-coupled systems. Therefore, to simplify practical calculations, the density operator $\sigma$ can be expressed as a linear combination of base operators $B_s$: (e.g., [47])

$$\sigma(t) = \sum_s b_s(t)B_s$$  \hspace{1cm} (2.12)

to transform the evolution of $\sigma$ into the evolution of these base operators. Providing both physical insight and computational convenience, product operators have been widely used in NMR simulations [47, 51-61]. Product operators are defined as products over the N spin-1/2 nuclei [47]:

$$B_s = 2^{(s-1)} \prod_{k=1}^{N} (I_{k\nu})^{\gamma_{s_d}}$$  \hspace{1cm} (2.13)
where \( k \) is an index of nucleus, \( v = x, y \) or \( z \), \( q \) = number of single-spin operators in the product, \( a_{sk} = 1 \) for \( q \) nuclei and \( a_{sk} = 0 \) for the \( N - q \) remaining nuclei.

The nomenclature of product operators is shown below. The one-spin operators are: [47]

- \( I_{kz} \): longitudinal magnetization of spin \( k \),
- \( I_{kx} \): in-phase \( x \)-magnetization of spin \( k \),
- \( I_{ky} \): in-phase \( y \)-magnetization of spin \( k \).

The two-spin operators are: [47]

- \( 2I_{kx}I_{lz} \) (\( v = x \) or \( y \)): \( x \) or \( y \)-magnetization of spin \( k \) antiphase with respect to spin \( l \),
- \( 2I_{kx}I_{ly} \): two-spin coherence of spins \( k \) and \( l \),
- \( 2I_{kz}I_{lz} \): longitudinal two-spin order of spins \( k \) and \( l \).

The three-spin operators are: [47]

- \( 4I_{kz}I_{lz}I_{mz} \): \( x \) or \( y \)-magnetization of spin \( k \), in antiphase with respect to the spins \( l \) and \( m \),
- \( 4I_{kx}I_{ly}I_{mz} \): two-spin coherence of spins \( k \) and \( l \), in antiphase with respect to spin \( m \),
- \( 4I_{kx}I_{lx}I_{mz} \): three-spin coherence,
- \( 4I_{kz}I_{lz}I_{mz} \): longitudinal three-spin order of spins \( k \), \( l \), and \( m \).

Only \( I_{kx} \) and \( I_{ky} \) induce observable signals in NMR.

**Evolution of product operators**

The evolution caused by a chemical shift at frequency \( \Omega_k \) is described by [47]:

\[
I_{kx} \rightarrow \frac{\Omega_{k} d}{\hbar} I_{kx} \cos(\Omega_{k} t) + I_{ky} \sin(\Omega_{k} t) \quad (2.14)
\]

and

\[
I_{ky} \rightarrow \frac{\Omega_{k} d}{\hbar} I_{ky} \cos(\Omega_{k} t) - I_{kx} \sin(\Omega_{k} t). \quad (2.15)
\]
The evolution caused by a coupling between two nuclei \( k \) and \( l \) within a network of coupled spins is described by [47]

\[
I_{kx} \rightarrow -\frac{\pi J_{kl} I_{kx}}{2} \rightarrow I_{kx} \cos(\pi J_{kl} t) + 2 I_{kx} I_{kz} \sin(\pi J_{kl} t) \tag{2.16}
\]

and

\[
I_{ky} \rightarrow -\frac{\pi J_{kl} I_{ky}}{2} \rightarrow I_{ky} \cos(\pi J_{kl} t) - 2 I_{ky} I_{kz} \sin(\pi J_{kl} t) \tag{2.17}
\]

Neglecting any off-resonance effects (i.e., the nutation axis of an RF pulse will be tilted away from the \( x-y \) plane when the \( B_1 \) frequency is set away from the Larmor frequency of the spins of interest), the evolution caused by an RF pulse with phase \( \phi \) and nutation angle \( \beta \) can be described by [47]

\[
I_{kx} - \beta [I_{kx} \cos \phi + I_{ky} \sin \phi] \rightarrow I_{kx} \cos \beta + I_{kx} \sin \beta \sin \phi - I_{ky} \sin \beta \cos \phi \tag{2.18}
\]

\[
I_{kx} - \beta [I_{kx} \cos \phi + I_{ky} \sin \phi] \rightarrow -I_{kx} \sin \beta \sin \phi + I_{kx} \left( \cos \beta \sin^2 \phi + \cos^2 \phi \right) + I_{ky} \sin^2 \beta (\cos 2 \phi) \tag{2.19}
\]

and

\[
I_{ky} - \beta [I_{kx} \cos \phi + I_{ky} \sin \phi] \rightarrow I_{kx} \sin \beta \cos \phi + I_{kx} \sin^2 \beta \cos \phi + I_{ky} \sin \beta \cos \phi + I_{ky} \sin^2 \phi \tag{2.20}
\]

The effect of a magnetic gradient on \( I_{kx} \) and \( I_{ky} \) can be described by

\[
I_{kx} (z_1) \rightarrow \gamma gz_1 \delta \rightarrow I_{kx} (z_1) \cos (\gamma gz_1 \delta) + I_{ky} (z_1) \sin (\gamma gz_1 \delta) \tag{2.21}
\]

and

\[
I_{ky} (z_1) \rightarrow \gamma gz_1 \delta \rightarrow I_{ky} (z_1) \cos (\gamma gz_1 \delta) - I_{kx} (z_1) \sin (\gamma gz_1 \delta) \tag{2.22}
\]

where \( I_{kx}(z_1) \) and \( I_{ky}(z_1) \) are \( x \) and \( y \) magnetizations at position \( z_1 \) along the \( z \)-axis. The final magnetization after a whole pulse sequence can be obtained by integrating the
magnetization over the length, $L$, of the (cylindrical) sample:

$$
\int_{-L/2}^{L/2} \left( a I_x + b I_y \right) dz ,
$$

(2.23)

where $a$ and $b$ are the coefficients for $I_x$ and $I_y$, respectively.

**Coherence level**

Product operators with $q_t$ transverse single spin operators $I_{kv} (v = x, y)$ are composed of MQ coherences of levels $p = q_t - 2n$ ($-q_t \leq p \leq q_t$, $n = 0, 1, 2, \ldots$) [47]. For example, $p = -1$ and $1$ for $I_{kv}$ and $I_{ky}$, $p = 0$ for $I_{kz}$, and $p = -2$, $0$, and $2$ for $2I_{kx}I_{ky}$.

**Evolution of product operators for the Hahn spin-echo and stimulated echo sequences**

The Hahn spin-echo [62] (Figure 2–5A) and stimulated echo (STE) [62] (Figure 2–5B) sequences are the skeletons for building PGSE NMR diffusion sequences so it is worthwhile to go through the evolution of product operators for these sequences.

For the Hahn spin-echo sequence [62] (Figure 2–5A), the evolution of product operators is

$$
I_z \xrightarrow{90^\circ_{z}} I_x \sin(\phi) - I_y \cos(\phi) \xrightarrow{\pi t_2} I_x \sin(\phi + 2\pi \Omega \tau) - I_y \cos(\phi + 2\pi \Omega \tau) \\
\xrightarrow{180^\circ_{z}} I_x \sin(\phi + 2\pi \Omega \tau - 2\phi_2) + I_y \cos(\phi + 2\pi \Omega \tau - 2\phi_2) \\
\xrightarrow{\pi t_2} I_x \sin(\phi - 2\phi_2) + I_y \cos(\phi - 2\phi_2). 
$$

(2.24)
Figure 2–5 The Hahn spin-echo [62] (A) and stimulated echo [62] (STE) (B) sequences. $\phi_1$, $\phi_2$, and $\phi_3$ are pulse phases. In a STE experiment, only the echo signal is selected by the use of ‘phase cycling’.

As shown in Eq. (2.24), the spin-echo forms at the end of the second $\tau$ period. Here only the $I_y$ term is marked as “Echo” because $I_x$ and $I_y$ are just two components of one coherence and the same is done for the STE sequence.
For the STE sequence [62] (Figure 2–5B), the evolution of product operators is

\[
I_x \xrightarrow{90_\phi^\circ} I_x \sin(\phi_1) - I_y \cos(\phi_1) \xrightarrow{\Omega_{\tau_1} I_x} I_x \sin(\phi_1 + 2\pi \Omega \tau_1) - I_y \cos(\phi_1 + 2\pi \Omega \tau_1)
\]

\[
\xrightarrow{90_\phi^\circ} \xrightarrow{\Omega_{\tau_1} I_x} \xrightarrow{90_\phi^\circ} \xrightarrow{\Omega_{\tau_1} I_x} \xrightarrow{90_\phi^\circ} \xrightarrow{\Omega_{\tau_1} I_x} \xrightarrow{90_\phi^\circ}
\]

\[
I_x \left( \frac{1}{2} \sin(\phi_1 - \phi_2 - \phi_3) - \frac{1}{2} \sin(4\pi \Omega \tau_1 + \phi_1 - \phi_2 + \phi_3) ight)
+ \frac{1}{4} \sin(4\pi \Omega \tau_1 - 2\pi \Omega \tau_2 + \phi_1 - 2\phi_2 + 2\phi_3)
- \frac{1}{4} \sin(2\pi \Omega \tau_2 - \phi_1 + 2\phi_2)
+ \frac{1}{4} \sin(2\pi \Omega \tau_2 + \phi_1 - 2\phi_3)
+ \frac{1}{4} \sin(4\pi \Omega \tau_1 + 2\pi \Omega \tau_2 + \phi_1)
\]

\[
+ I_y \left( \frac{1}{2} \cos(\phi_1 - \phi_2 - \phi_3) + \frac{1}{2} \cos(4\pi \Omega \tau_1 + \phi_1 - \phi_2 + \phi_3) \right)
- \frac{1}{4} \cos(4\pi \Omega \tau_1 - 2\pi \Omega \tau_2 + \phi_1 - 2\phi_2 + 2\phi_3)
+ \frac{1}{4} \cos(2\pi \Omega \tau_2 - \phi_1 + 2\phi_2)
+ \frac{1}{4} \cos(2\pi \Omega \tau_2 + \phi_1 - 2\phi_3)
- \frac{1}{4} \cos(4\pi \Omega \tau_1 + 2\pi \Omega \tau_2 + \phi_1)
\]

\[
+ I_z \left( \frac{1}{2} \cos(\phi_3 - 2\pi \Omega \tau_2 + \phi_1 + 2\pi \Omega \tau_1 - 2\phi_2) 
- \frac{1}{2} \cos(-\phi_3 + 2\pi \Omega \tau_2 + \phi_1 + 2\pi \Omega \tau_1) \right). \tag{2.25}
\]

As shown in Eq. (2.25), two STE coherences, stimulated echo and stimulated anti-echo (or STE#1 and STE#2 as referred to by Kingsley [55]), form at the end of the second \(\tau_1\) period and the other four unwanted coherences can be removed by applying
pulsed gradients during the $\tau_2$ period. By the use of phase cycling and/or pulsed gradients (See Figure 2–6B), one of the two STE coherences can be selected and normally the echo, which has opposite coherence levels in the two $\tau_1$ periods, is selected. For example, the echo is selected when two identical pulsed gradients are applied in the first and second $\tau_1$ periods, respectively, while the anti-echo is selected when two identical pulsed gradients but with opposite polarities are applied in the first and second $\tau_1$ periods, respectively.

2.2 Fundamentals of PGSE NMR

Spin phase shifts caused by the application of magnetic field gradients are analyzed to demonstrate the effects of diffusion on spin phase shift distribution along the direction of the applied gradients. However, the correlation between the diffusion coefficient and the spin-echo attenuation follows Stejskal and Tanner analysis [63] based on solving the Bloch-Torrey equations [64] because the pulse sequence development in this thesis is based on Stejskal and Tanner analysis.

For a single spin, the cumulative phase shift caused by a single magnetic field gradient oriented along $z$-direction can be calculated by (e.g., [20])

$$\phi(t) = \gamma p(t') \left( E_0 + \int_0^t g(t') z(t') dt' \right), \quad (2.26)$$

where $p(t')$ is the coherence level (with a value of -1, 0, or 1) in one of the selected coherence pathways at time $t'$. For a MQ coherence, $\gamma_{\text{eff}}(t')(p(t')/|p(t')|)$ should be substituted for $\gamma p(t')$ in Eq. (2.26).
Figure 2–6 The Hahn spin-echo PGSE sequence (A) and the stimulated echo-based PGSE sequence (STE-based PGSE) (B). For the Hahn spin-echo PGSE sequence, two pulsed gradients of duration $\delta$ and magnitude $g_a$ are inserted into each $\tau$ delay; for the STE-based PGSE sequence, two pulsed gradients of duration $\delta$ and magnitude $g_a$ are applied in the two encoding intervals, (0 to $t_1$) and ($t_1 + t_2$ to $2t_1 + t_2$), which are separated by the phase storage period ($t_2$). The background gradient has a magnitude of $g_0$. The separation between the leading edges of the pulsed gradients is denoted by $\Delta$ (i.e., observation time or diffusion time). The applied gradient is along the $z$-axis (the direction of the static magnetic field, $B_0$). The Hahn spin-echo-based PGSE sequence can be divided into 6 intervals according to the variation of the net magnetic gradients. Only the second half of the echo is digitized and used as the free induction decay (FID). The coherence pathways are also shown here. The STE-based PGSE sequence only captures half the magnetization as the Hahn spin-echo PGSE sequence does.
By evaluating Eq. (2.26) over the first $\tau$ period of the original Hahn spin-echo PGSE sequence (Figure 2–6A), the phase shift of a spin $i$ with a single quantum coherence in a spin ensemble at time $\tau$ is obtained as (e.g., [20]),

$$
\phi_i(t) = \gamma B_0 \tau + \gamma g \int_{t_i}^{t_i+\Delta} z_i(t) \, dt,
$$

(2.27)

where the first term is the phase shift due to $B_0$, and the second one is due to the gradient. At the end of the first $\tau$ period, the coherence level is changed from 1 to −1 by the application of $\pi$ pulse and thus, based on Eq. (2.26), if there is no diffusion, the phase shift generated by the first gradient is completely cancelled by the phase shift generated by the second gradient. However, if the spin does diffuse between the two gradients, the net phase shift experienced by spin $i$ at the end of the sequence is given by [20]

$$
\phi_i(2\tau) = \left(\gamma B_0 \tau + \gamma g \int_{t_i}^{t_i+\Delta} z_i(t) \, dt\right) - \left(\gamma B_0 \tau + \gamma g \int_{t_i+\Delta}^{t_i+\Delta+\delta} z_i(t') \, dt'\right)
$$

(2.28)

$$
= \gamma g \int_{t_i}^{t_i+\Delta} z_i(t) \, dt - \gamma g \int_{t_i+\Delta}^{t_i+\Delta+\delta} z_i(t') \, dt'.
$$

For an ensemble of diffusing nuclei, the intensity of the echo signal at $t = 2\tau$ is given by (e.g., [20])

$$
S(2\tau) = S^{i=0} \exp \left( -\frac{2\tau}{T_2} \right) \int_{-\infty}^{\infty} P(\phi, 2\tau) e^{i\phi} d\phi,
$$

(2.29)
where \( S^{t=0} \) is the signal immediately after the first \( \pi/2 \) pulse (i.e., initial excitation in the PGSE sequence) and \( P(\phi, 2\tau) \) is the (relative) phase-distribution function.

Similarly for the STE-based PGSE sequence, the result is

\[
S(2\tau_1 + \tau_2) = \frac{S^{t=0}}{2} \exp \left( -\frac{2\tau_1}{T_2} \right) \exp \left( -\frac{\tau_2}{T_1} \right) \int_{-\infty}^{\infty} P(\phi, 2\tau_1 + \tau_2) e^{i\phi} d\phi. \tag{2.30}
\]

Comparing Eqs. (2.29) and (2.30) and considering \( \tau >> \tau_1 \), it is clear that the STE-based PGSE sequence is less susceptible to the attenuation due to transverse relaxation than the Hahn spin-echo PGSE sequence, which makes it suitable for NMR diffusion experiments on samples with fast transverse relaxation (e.g., macromolecules such as proteins and polymers). It will be shown in the following discussions that the STE-based PGSE sequence also suffers less attenuation due to background gradients. However, it is also shown in

Figure 2–6 that the STE-based PGSE sequence captures only half of the total magnetization while the Hahn spin-echo PGSE sequence captures all. Therefore, in practice there is always a trade-off between the signal loss due to the use of STE-based sequence and the signal loss due to relaxation and background gradients.

Based on different descriptions of diffusion, the spin-echo attenuation caused by diffusion can be calculated in different ways. Based on the MSD description, neglecting chemical exchange, the evolution of \( \mathbf{M} \) in the presence of diffusion and longitudinal and transverse relaxation can be described by (e.g., [64]):

\[
\frac{\partial \mathbf{M}(\mathbf{r}, t)}{\partial t} = \gamma \mathbf{M} \times \mathbf{B}(\mathbf{r}, t) - \frac{M_i^z + M_j^z}{T_2^*} - \frac{(M_z - M_0)}{T_1} + D \nabla^2 \mathbf{M}. \tag{2.31}
\]
The spin-echo attenuation caused by diffusion can be calculated via the Stejskal-Tanner equation (neglecting relaxation processes) [63],

\[
\ln \left[ \frac{S_{t=acq}}{S_{t=0}} \right] = -D \int_0^t F^2(t'')dt'',
\]

(2.32)

where \(F\) (rad m\(^{-1}\)) is the spin dephasing given by

\[
F(t'') = \int_0^{t''} \gamma_{\text{eff}}(t') \frac{p(t')}{|p(t')|} g(t') dt',
\]

(2.33)

\(S_{t=acq}\) is the echo signal, \(S_{t=0}\) is the signal immediately after the first \(\pi/2\) pulse (i.e. initial excitation in the PGSE sequence), \(t\) (s) is the time at which the signal acquisition begins, and \(g\) (T m\(^{-1}\)) is the net gradient (the sum of all magnetic gradients existing in the system). As shown in Figure 2–6A, the Hahn spin-echo-based PGSE sequence can be divided into 6 intervals according to the variation of \(g\).

Eq. (2.32) is evaluated over each period of a pulse sequence. As an example, for the Hahn spin-echo PGSE sequence shown in Figure 2–6A, the spin-echo attenuation in the presence of background gradients is derived as
\[
\ln \left[ \frac{S^{\text{acq}}}{S^{\text{ref}}} \right] = -D \int_0^L \gamma^2 \left[ \int_0^{t_1} (-g_a) dt' \right]^2 dt^* + \int_{t_1}^{t_2} \gamma^2 \left[ \int_0^{t_1} (-g_a + g_s) dt' + \int_{t_1}^{t_2} g_s dt' \right]^2 dt^*
+ \ldots
\]

\[
+ 2 \int_{t_1}^{t_2} \gamma^2 \left[ \int_0^{t_1} (-g_a) dt' + \int_{t_1}^{t_2} (g_a - g_\alpha) dt' + \int_{t_1}^{t_2} g_\alpha dt' \right]^2 dt^*
\]

\[
= -\gamma^2 D \left[ g^2 \sigma^2 \left( \frac{\Delta - \delta}{3} \right) + g_a g_\alpha \delta \left[ 2 \tau^2 - t_1^2 - t_2^2 - \delta(t_1 + t_2) - \frac{2}{3} \delta^2 \right] + \frac{2 \tau^3}{3} g^2 \right]
\]

\[
= -bD,
\]

where \( b \) is normally called the attenuation factor.

Similarly for the STE-based PGSE sequence (Figure 2–6B), the result is

\[
\ln \left[ \frac{S^{\text{acq}}}{S^{\text{ref}}} \right] = -\gamma^2 D \left[ g^2 \sigma^2 \left( \frac{\Delta - \delta}{3} \right) + g_a g_\alpha \delta \left[ 2 \tau^2 - t_1^2 - t_2^2 - \delta(t_1 + t_2) - \frac{2}{3} \delta^2 \right] + \frac{2 \tau^3}{3} g^2 \right]
\]

\[
= -bD,
\]

An alternative method for relating the spin-echo attenuation with \( D \) has been presented by Stepišnik [65]. This approach is based on the VCF description, and the effect of the gradients in PGSE experiments can be treated via the accumulated spin dephasing. Hence, the spin-echo attenuation can be related to magnetic gradients by [66]

\[
\ln \left[ \frac{S^{\text{acq}}}{S^{\text{ref}}} \right] = -\frac{1}{2} \int_0^L \int \left[ F(t_1) \langle v_1(t_1) v_2(t_2) \rangle F(t_2) dt_1 dt_2 \right]. \quad (2.36)
\]
Using Eq. (1.13), this can be rewritten as

$$
\ln \left[ \frac{S^{\text{sat} = q}}{S^{\omega = 0}} \right] = -\frac{1}{\pi} \int_0^\infty D(\omega) |F'(\omega)|^2 d\omega , \quad (2.37)
$$

where $F'(\text{rad s m}^{-1})$ is the dephasing spectrum, and is defined by

$$
F'(\omega) = \int_0^t F(t^*) e^{i\omega t^*} dt^*. \quad (2.38)
$$

From Eq. (2.37), it can be seen that the spectrum of the velocity autocorrelation is “probed” by a sampling function given by the frequency spectrum of spin dephasing [66].

At low frequencies ($D = D(\omega = 0)$), Eq. (2.37) can be written as (e.g., [67])

$$
\ln \left[ \frac{S^{\text{sat} = q}}{S^{\omega = 0}} \right] = -\frac{1}{\pi^2} D \int_0^\infty |F'(\omega)|^2 d\omega . \quad (2.39)
$$

If Parseval’s theorem (e.g., [68]) is taken into account

$$
\frac{1}{\pi^2} \int_0^\infty |F'(\omega)|^2 d\omega = \int_0^t F^2(t^*) dt^* . \quad (2.40)
$$

Consequently, Eq. (2.39) is equivalent to Eq. (2.32) at low frequencies.

In the presence of background gradients, $F'$ can be written as [67, 69]
\[ F' = F'_0 + F'_a, \] (2.41)

where \( F'_0 \) is the dephasing spectrum of background gradients and \( F'_a \) is the dephasing spectrum of the applied gradients, and thus the spin-echo attenuation is given by (e.g., [67])

\[
\ln \left[ \frac{S_{t=\text{acq}}}{S_{t=0}} \right] = -\frac{1}{\pi} D \int_0^\infty \left[ \left| F'_a \right|^2 + 2 \Re \left( F'_a F'_0^* \right) + \left| F'_0 \right|^2 \right] d\omega. \tag{2.42}
\]

The amplitude of the cross-term of \( F'_0 \) and \( F'_a \) in Eq. (2.42) is determined by the overlap of \( F'_0 \) and \( F'_a \). As shown by the simulations using some typical experimental parameters (Figure 2–7), there is significant overlap between the \( F'_0 \) and \( F'_a \) for a Hahn spin-echo-based PGSE sequence in Figure 2–6A. This is the explanation of the deleterious effects of background gradients in the PGSE experiments based on the VCF description.
Figure 2–7 The dephasing spectra of the applied gradients \( \frac{|F_a|}{\gamma g_a \tau^2} \) and the background gradients \( \frac{|F_0|}{\gamma g_0 \tau^2} \) for the Hahn spin-echo-based PGSE sequence shown in Figure 2–6A. The spectra were calculated using Eq. (2.38) with \( \delta = 1.7 \text{ ms} \), \( t_1 = 5.1 \text{ ms} \), \( \tau = 54 \text{ ms} \) and \( \Delta = 100 \text{ ms} \). The curves are normalized with respect to the maximum values.
Chapter 3  Review of Advanced PGSE Methods

3.1 Background Gradient Suppression PGSE Methods

It has long been realized that the existence of background gradients (also referred to as internal gradients or static gradients \( g_0 \)) caused by the imperfect shimming of \( B_0 \) and/or differences in magnetic susceptibility both within and around the sample can cause deleterious effects on the determination of diffusion and this area has recently been reviewed by Zheng and Price [70]. Therefore, background gradient suppression PGSE sequences have been intensively studied [23, 40, 43, 46, 67, 69-84].

3.1.1 Effects of Background Gradients

All spins having the same diffusion coefficient in the presence of a constant \( g_0 \)

Simplistically it can be assumed that every spin in the sample experiences exactly the same chemical and physical environment and thus has the same diffusion coefficient and \( g_0 \) stays constant through the whole pulse sequence. This simplistic model facilitates our conceptual understanding of the deleterious effects of background gradients on PGSE experiments.

In practice, the \( S^{t=\text{acq}} \) is normalized against the echo signal acquired in the absence of applied pulsed gradients \( (S^{t=\text{acq}}_{g=0}) \) instead of the signal right after the initial \( \pi/2 \) pulse \( (S^{t=0}) \) and Eq. (2.34) becomes
\[ \ln \left[ E \right] = \ln \left[ \frac{S_{t=acq}}{S_{t=acq}^{g_s=0}} \right] = -\gamma^2 D \left[ g_s^2 \delta^2 \left( \Delta - \frac{\delta}{3} \right) + g_s g_0 \delta \left[ 2r^2 - t_1^2 - t_2^2 - \delta(t_1 + t_2) - \frac{2}{3}\delta^2 \right] \right] \]  

where the terms are defined in Figure 2–6. Since \( S_{t=acq}^{g_s=0} \) includes the effects of the \( g_0 \) term and relaxation, the \( g_0 \) term is normalized out in Eq. (3.1), and for a single diffusing species, the effects of relaxation is also normalized out. In a PGSE experiment, it is common to vary the intensity of the applied pulsed gradient \((g_a)\) with \( \Delta \) made suitably long. With \( g_a \) being varied, the cross-term may hamper the precise determination of the diffusion coefficient since \( g_0 \) is unknown. To illustrate the deleterious effects of the cross-term, some simulations for a Hahn spin-echo PGSE experiment on a water sample in the presence of background gradients have been performed. Assuming \( D' \) is the determined diffusion coefficient and \( D \) is the real diffusion coefficient, the relative error can be calculated by

\[ \frac{D' - D}{D} = \frac{g_0}{g_a} \cdot \frac{2r^2 - t_1^2 - t_2^2 - \delta(t_1 + t_2) - \frac{2}{3}\delta^2}{\delta \left( \Delta - \frac{\delta}{3} \right)} \]  

\[ = f \left( \frac{g_0}{g_a} \right). \]  

As shown in Figure 3–1, the error increases when \( r/\delta \) and/or \( g_0/g_a \) increases. For a typical Hahn spin-echo PGSE experiment (e.g. \( r/\delta = 50 \)), \( g_0 \) with a strength of only 2% of the strength of \( g_a \) can cause more than 100% error in the determination of the diffusion coefficient.
Figure 3–1 A plot of $(D' - D)/D$ versus $\tau/\delta$ and $g_0/g_a$. The simulation was performed using Eq. (3.2) with $\delta = 2$ ms and $t_1 = t_2 = 1$ ms.

Similarly for the STE-based PGSE sequence, the spin-echo attenuation can be written as

$$
\ln[E] = -\gamma^2 D \left\{ \frac{g_0^2}{g_a^2} \delta^2 \left( A - \frac{\delta}{3} \right) + g_a g_0 \delta \left[ 2\tau_1 \tau_2 + 2\tau_1^2 - \frac{2}{3} \delta^2 - \delta (\delta_1 + \delta_2) - \left( \delta_1^2 + \delta_2^2 \right) \right] \right\}, \quad (3.3)
$$

where the terms are defined in Figure 2–6. The effects of background gradients on STE-based PGSE experiments are similar to those shown in Figure 3–1.

As mentioned at the beginning of this Chapter, how the background gradients affect the determination of diffusion is far more complicated than the simplified example given above as in practice not all spins have the same diffusion coefficient
and $g_0$ is always non-constant both spatially and temporally. Therefore, the
determined diffusion coefficients are always lower than the real diffusion coefficients
in the presence of background gradients due to the ensemble averaging of the
inhomogeneous diffusion and the effects of inhomogeneous background gradients.
These ensemble averaging effects are discussed in the following Sections.

**Ensemble average over spins with different diffusion coefficients**

The derivation of Eq. (3.2) is based on the assumption that every spin in the sample
diffuses with the same diffusion coefficient. However, in reality, spins at different
positions in the sample may have different diffusion coefficients due to the
differences in local viscosities and temperatures. To illustrate the effects of the
averaging, the spins have been divided into two equal groups, a fast-diffusing group
and a slowly-diffusing group. Here, it has been assumed that the MSD of spins on the
time scale of PGSE sequences is far smaller than the size of inhomogeneity which
causes differences in diffusion. Therefore, the attenuated echo signal is given by

$$S = S_{\text{fast}} + S_{\text{slow}},$$  \hspace{1cm} (3.4)

where $S_{\text{fast}}$ is the echo attenuation contributed by the fast-diffusing spins and $S_{\text{slow}}$ is
the echo attenuation contributed by the slowly-diffusing spins. The attenuated echo
signals can be correlated with the signal immediately after the initial excitation ($S_0$)
by (neglecting the effects of transverse relaxation)

$$S_{\text{fast}} = S_0 e^{-\left(h_t + h_{\text{reso}} + h_{\text{ps}}\right)D_{\text{fast}}}$$  \hspace{1cm} (3.5)

and
Based on Eq. (3.4), (3.5) and (3.6), the attenuation can be calculated by

$$E = \frac{S}{S_{g=0}} = \frac{e^{-b_0 D_{\text{int}}} + e^{-b_0 D_{\text{slow}}}}{e^{-b_0 D_{\text{int}}} + e^{-b_0 D_{\text{slow}}}}. \quad (3.7)$$

If there is no background gradient (i.e., \(b_{\text{cross}} = 0\) and \(b_{g_0} = 0\)), Eq. (3.7) becomes

$$E = \frac{e^{-b_0 D_{\text{int}}} + e^{-b_0 D_{\text{slow}}}}{2}. \quad (3.8)$$

As shown in Eq. (3.8), the coefficient in front of the fast-diffusion attenuation term is the same as that in front of the slow-diffusion attenuation term.

If \(g_0 \neq 0\), on the other hand, the attenuation should be calculated by Eq. (3.7), which can be rearranged to

$$E = Ae^{-b_0 D_{\text{int}}} + Be^{-b_0 D_{\text{slow}}}, \quad (3.9)$$

where

$$A = \frac{e^{-(b_{\text{cross}} + b_{g_0})D_{\text{int}}}}{e^{-b_0 D_{\text{int}}} + e^{-b_0 D_{\text{slow}}}} \quad (3.10)$$

and
\[ B = \frac{e^{-\left(h_{\text{cross}}+b_{G}\right)d_{\text{low}}}}{e^{-b_{G}d_{\text{low}}} + e^{-h_{G}d_{\text{low}}}}. \] (3.11)

Since \( A < B \) and \( A + B < 1 \) (NB \( b_{\text{cross}} > 0 \) and \( b_{G > 0} \)), in the presence of background gradients, a smaller \( E \) and thus a smaller measured diffusion coefficient will be obtained.

**Ensemble averaging of the effects of inhomogeneous background gradients**

The inhomogeneous background gradients are normally generated by the magnetic susceptibility inhomogeneity inside and/or around the sample with complicated structures in terms of magnetic susceptibility. Thus, spins inside different local structures may experience different background gradients. When the MSD’s of diffusing spins become comparable with the inhomogeneity of background gradients, the background gradients experienced by the spin ensemble will start to fluctuate through time. The effects of rapidly varying background gradients can be treated as a mechanism accelerating transverse relaxation [43, 85]. More slowly varying background gradients can be treated as a distribution \( f(g_0) \) [86], for instance, a Gaussian distribution [85]

\[ f(g_0) = \exp\left(-g_0^2/2\sigma^2\right)/\left(2\pi\sigma^2\right)^{1/2}, \] (3.12)

where \( \sigma^2 \) stands for the variance of the background gradients. If it is assumed that during the diffusion time the background gradient experienced by a single spin is constant but different spins see different background gradients (NB with a distribution of \( f(g_0) \)), the normalized echo attenuation is determined to be [85]
\[
\langle \ln[E]\rangle = -(\gamma \delta g)^2 (\Delta - \delta/3) D \left[ 1 - 0.5 \gamma^2 \Delta \sigma^2 D \left( 2\tau - \Delta/2 \right)^2 \right], 
\]

and the determined diffusion coefficient can be written as [85]

\[
D' = D \left[ 1 - 0.5 \gamma^2 \Delta \sigma^2 D \left( 2\tau - \Delta/2 \right)^2 \right].
\]

### 3.1.2 Estimation of the Amplitude of Background Gradients

Although the existence of background gradients in NMR systems is almost unavoidable, the effects of background gradients are usually ignored because the quality of the experimental data is normally judged by looking at how well the attenuation function can fit the data and discrepancies are only evident for large background gradients.

In a series of PGSE diffusion experiments on a water sample in the presence of the background gradients generated by using the bad shimming of the magnet, it was found that even though the line width increased and the determined diffusion coefficient decreased with the increasing strength of the background gradients, there was no significant changes in the standard errors of the measured diffusion coefficients. Therefore, before each PGSE experiment, the magnitude of background gradients should be carefully estimated.

Estimations of the amplitudes of background gradients can be classified as qualitative or quantitative. Qualitative methods are normally used to check if there are any deleterious effects of background gradients in the PGSE experiments on relatively magnetically homogeneous samples (e.g., 1 mM glucose in pure $^2\text{H}_2\text{O}$), and quantitative methods are normally used to determine the amplitudes of background
gradients in magnetically inhomogeneous samples (e.g., packed polymer or glass spheres filled with water).

**Qualitative measurements**

In a PGSE experiment on a relatively magnetically homogeneous sample, the easiest way to check if there are any deleterious effects of background gradients is to perform a standard Hahn spin-echo PGSE experiment (Figure 2–6) on the sample followed by the same experiment but with gradient pulses of the opposite polarity (e.g., [41]), for example, varying the $z$-gradient strength from 0.03 to 0.51 T m$^{-1}$ in one experiment and varying the $z$-gradient strength from -0.03 to -0.51 T m$^{-1}$ in the other (here the minus sign means the inversion of the direction of the gradients (i.e., $+z \rightarrow -z$)). As shown in Eq. (3.1), if $g_0$ is significant, the inversion of the sign of $g$ will invert the sign of the cross-term and thus cause changes in attenuations and determined diffusion coefficients, whereas if $g_0$ is negligible, the inversion will make no difference. Therefore, if the difference between the two determined diffusion coefficients is statistically significant, background gradients might be affecting the experiments.

**Quantitative measurements**

For a typical magnetically inhomogeneous sample (e.g., packed glass beads filled with water), the full width at half maximum (FWHM) varying from a few hundred to a few thousand Hertz is normally more than one hundred times that of a magnetically homogeneous sample (e.g., 1–50 Hz). The significant line broadening caused by large background gradients makes $T_2 >> T_2^*$ valid; therefore, the amplitude of background gradients can be estimated by (e.g., [82, 87])
\[ g_0 = \frac{2\pi \Delta \nu}{\gamma d_{\text{mean}}} \]  

(3.15)

where \( \Delta \nu \) (Hz) is the FWHM of the Fourier-transformed NMR signal from the liquid inside the sphere pack and \( d_{\text{mean}} \) (m) is the mean diameter of the spheres.

### 3.1.3 Suppression of the Effects of Background Gradients

**Suppression of \( g_0 \) terms**

In 1954, Carr and Purcell developed the Carr-Purcell \( \pi \)-pulse train sequence to suppress the effects of background gradients in \( T_2 \) experiments [88]. The basic idea of this sequence is to use a \( \pi \)-pulse train to divide the experimental period into small intervals so that the spin-dephasing due to background gradients in one interval is cancelled by the spin-dephasing in the following interval. However, for the Carr-Purcell sequence, the calibration of the \( \pi \) pulses is crucial and a small error soon propagates. Therefore, Meiboom and Gill [73] introduced the Carr-Purcell-Meiboom-Gill (CPMG) sequence (Figure 3–2), in which the phase of the \( \pi/2 \) pulse is shifted by 90° relative to the phase of the \( \pi \) pulses. The spin-echo attenuation of the CPMG sequence can be written as

\[
\ln \frac{S^{\text{aq}}}{{S^{\text{aq}}}} = -\frac{\gamma^2 D g_0^2 T^3}{12n^2},
\]  

(3.16)

where \( n \) is the number of \( \pi \) pulses and \( T = 2n\tau \). As shown by Eq. (3.16), when a large number of \( \pi \) pulses are inserted into the experimental period, the spin-echo attenuation due to the \( g_0 \) term is greatly reduced.
Suppression of cross-terms

According to Eq. (2.34) and Eq. (2.35), there are different approaches suppressing the deleterious effects of background gradients. The simplest is to keep the cross-term constant during the experiment so that the echo attenuation will not “feel” the existence of the cross-term. One successful application of this method was performed by integrating an \( n \)-\( \pi \)-pulse train sequence with two fixed-amplitude applied pulsed gradients (Figure 3–3) [40]. The spin-echo attenuation of this sequence can be written as

\[
\ln[E] = -\gamma^2 D \left\{ g_a^2 \delta^2 (\Delta - \delta/3) \right. \\
\left. - g_a g_o \delta \left[ (t_1^2 + t_3^2) + \delta (t_1 + t_3) + \frac{2}{3} \delta^2 - 2\tau^2 \right] \right\}, \tag{3.17}
\]

where \( t_3 = \tau - t_1 - \delta \). Importantly, only the \( g_a \) term in Eq. (3.17) includes \( \Delta \). When the diffusion coefficient is determined, the duration of \( \Delta \) is changed by placing the applied gradients in different \( \tau \)-intervals, while \( g_a, \delta, t_1, t_3, \) and \( \tau \) are held constant so that the cross-term remains constant. With the echo attenuation as the dependent variable and \( \Delta \) as the independent variable, the true diffusion coefficient is obtained by regressing Eq. (3.17) onto the experimental data.
Figure 3–3 The $n\pi$-pulse CPMG sequence with two fixed-amplitude pulsed gradients in the presence of background gradient. $\Delta = (2m - 1) \tau$, $m = 1, 3, 5, 7, 9……$

Table 3-1 Cross-terms in different time intervals for Hahn spin-echo PGSE sequences (Figure 2–6 and Figure 3–4)

<table>
<thead>
<tr>
<th>Cross-term $\propto (-\gamma^2 D \delta g_x g_0)^T$</th>
<th>Original Hahn spin-echo sequence</th>
<th>Double-echo sequence</th>
<th>Double-echo sequence with bipolar gradients $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to $\tau/2$</td>
<td>$t_1^2/4 - t_1^2 - t_2 \delta - \delta^2/3$</td>
<td>$t_1^2/4 - t_1^2 - t_2 \delta - \delta^2/3$</td>
<td>$(t_1 + t_3 + t_5/2) + (t_1/4 + t_3/2) \delta + \delta^2/12$</td>
</tr>
<tr>
<td>$\tau/2$ to $\tau$</td>
<td>$3t_1^2/4$</td>
<td>$t_1^2/4$</td>
<td>$(t_1^2 + t_1 t_3 + t_3^2/2) + (3t_1/4 + t_3/2) \delta + \delta^2/6$</td>
</tr>
<tr>
<td>$\tau$ to $3\tau/2$</td>
<td>$3t_1^2/4$</td>
<td>$-t_1^2/4$</td>
<td>$-(t_1^2 + t_1 t_3 + t_3^2/2) - (3t_1/4 + t_3/2) \delta - \delta^2/6$</td>
</tr>
<tr>
<td>3$t\tau/2$ to $2\tau$</td>
<td>$t_1^2/4 - t_2^2 - t_2 \delta - \delta^2/3$</td>
<td>$-t_1^2/4 + t_2^2 + t_2 \delta + \delta^2/3$</td>
<td>$-(t_1 t_3 + t_3^2/2) - (t_1/4 + t_3/2) \delta - \delta^2/12$</td>
</tr>
<tr>
<td>0 to $2\tau$</td>
<td>$2t_1^2 - ( t_1^2 + t_2^2) - \delta(t_1 + t_2)$</td>
<td>$(t_1 + t_2 + \delta)(t_2 - t_1)$</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Pulsed gradients are placed around $\pi$ pulses symmetrically.
Table 3-2 Cross-terms in different time intervals for STE-based PGSE sequences (Figure 2–6 and Figure 3–5)

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Original STE based sequence</th>
<th>9-interval sequence</th>
<th>13-interval sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to $t_1$</td>
<td>$\delta_2^2 + 2\delta_1\delta_2$</td>
<td>$-(\delta_2^2 + \delta_2^3/3)$</td>
<td>$-(2\delta_1 + 2\delta_2 + \delta)(\delta_1 + 3\delta_2 + \delta)/4$</td>
</tr>
<tr>
<td>$t_1$ to $t_2 + t_1$</td>
<td>$2\tau^2(\delta_1 + \delta_2 + \delta)$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$t_2 + t_1$ to $t_2 + 2\tau_1$</td>
<td>$\delta_1^2 + 2\delta_1\delta_2$</td>
<td>$\delta_1^2 + \delta_1\delta + \delta^2/3$</td>
<td>$(2\delta_1 + 2\delta_2 + \delta)(3\delta_1 + \delta_2 + \delta)/4$</td>
</tr>
<tr>
<td>0 to $t_2 + 2\tau_1$</td>
<td>$2\tau_1\tau_2 + 2\tau_1^2 - 2\delta^2/3$</td>
<td>$\tau_1(\delta_1 - \delta_2)/2$</td>
<td>$\tau_1 (\delta_1 - \delta_2)/2$</td>
</tr>
</tbody>
</table>

$^a t_1 = \delta_1 + \delta_2 + \delta.$
$^b \tau_1 = 2(\delta_1 + \delta_2 + \delta)$
$^c \tau_1 = 2\delta_1 + 2\delta_2 + \delta$

The other way of suppressing the deleterious effects background gradients is to use special RF pulse and gradient pulse arrangements to reduce or eliminate the cross-term. To minimize the echo attenuation due to transverse relaxation and obtain a good signal-to-noise (S/N) ratio, PGSE diffusion experiments are normally performed by varying the strength of the applied gradients with $\Delta$ as short as possible. Therefore, the cross-terms have to be reduced or eliminated in these experiments.

Determining where the cross-terms come from is the first step in the development of the sequences for reducing or eliminating the cross-terms. For the original Hahn spin-echo PGSE sequence (Table 3-1), the cross-terms build up significantly during the interval $\tau/2 - 3\tau/2$ because the spin-dephasing caused by the background gradients has accumulated during the interval 0 - $\tau$. Similarly for the original STE-based PGSE sequence (Table 3-2), the cross-term has a sharp rise.
during the storage time due to the accumulation of spin-dephasing caused by background gradients in the encoding interval [72]. Thus, the first step to suppress the cross-terms is to minimize the spin-dephasing caused by background gradients during the interval $0 - \tau$ for the original Hahn spin-echo PGSE sequence or during the encoding interval for the original STE-based PGSE sequence. The simplest way to achieve this is using one additional $\pi$ pulse to divide the $0 - \tau$ interval or the encoding interval into two halves so that the spin-dephasing generated by background gradient in the first half can be cancelled in the second half. A double-echo PGSE sequence (Figure 3–4a) is the simplest modification of the original Hahn spin-echo PGSE sequence that can suppress the effects of background gradients. As shown in Table 3-1, there is no cross-term from the interval $\tau/2 - 3\tau/2$. More importantly, when $t_1 = t_2$, the cross-term from the first $\tau/2$ interval will be cancelled by the cross-term from the last $\tau/2$ interval, and thus the total cross-term will be eliminated. For the original STE-based PGSE sequence, the simplest modification is shown in Figure 3–5a [72]. Because of the insertion of the two additional $\pi$ pulses, the cross-term from the storage time is eliminated. Further, the cross-term from the encoding interval will be cancelled by the cross-term from the decoding interval when $\delta_1 = \delta_2$. 
Figure 3–4 (a) The double-echo PGSE sequence. (b) The double-echo PGSE sequence with bipolar-gradients (e.g., [71, 80]).

To enhance the spin dephasing (i.e., encoding) caused by the $g_a$ term, more gradients can be added into the sequences. By using a double-echo-based sequence with bipolar gradients (Figure 3–4b) (e.g., [71, 80]), the cross-term generated in the first $\tau$ interval is cancelled by the cross-term generated in the second $\tau$ interval when pulsed gradients are placed around $\pi$ pulses symmetrically [89] (Table 3-1). An STE-based PGSE sequence with bipolar gradients is shown in Figure 3–5b [72]. By using this sequence, the cross-term generated in the encoding interval is cancelled by the cross-term generated in the decoding interval when $\delta_1 = \delta_2$ (Table 3-2).
Figure 3–5 (a) The modified STE-based PGSE sequence. (This sequence can be divided into 9 intervals according to the variation of the effective gradients so it is also referred to as a 9-interval STE-based PGSE sequence [72].) (b) The STE-based PGSE sequence with bipolar gradients. (This sequence is also referred to as a 13-interval STE-based PGSE sequence [72].)

3.2 Solvent Suppression PGSE Methods

3.2.1 WATERGATE Sequence

The huge solvent signal can saturate the NMR receiver and thus cause insufficient amplification of weak signals from molecules of interest, overlap with signals of interest, and also cause baseline distortions. Numerous solvent suppression methods have been proposed and they can be classified either as pulse sequence methods or hardware/post-processing methods [90]. Pulse sequence methods can be loosely divided into three groups: saturation based, magnetization destruction based, and methods avoiding solvent saturation [90].

However, only a subset of these methods may be combined with PGSE diffusion sequences due to the deleterious effects of radiation damping and long range dipole-dipole interactions [90-92]. WATERGATE [93-95] is one of the most efficient and easily implemented suppression techniques which can be combined with PGSE
diffusion experiments. As shown in Figure 3–6, a typical WATERGATE sequence is like a short “\( \tau \)" version of Hahn spin-echo PGSE sequence with the hard (i.e., non-selective) \( \pi \) pulse replaced by a soft (i.e., selective) \( \pi \) pulse which inverts all the magnetizations except the solvent magnetizations (normally on-resonance). Without seeing the selective \( \pi \) pulse, the solvent magnetizations experiences the same coherence level and thus being destroyed due to the accumulated dephasing effects of the two pulsed gradients; the magnetizations of the molecules of interest are kept intact due to the dephasing effects of the two pulsed gradients cancelling each other. The essence of the WATERGATE sequence is “wanted and unwanted magnetizations experience different coherence pathways”.

Figure 3–6 A typical WATERGATE sequence. The vertical bar grouping stands for a selective \( \pi \) pulse, shaped gradients are used to minimize eddy current effects, and the typical settings for \( g_a \), the duration of each gradient, and \( \tau \) are 0.1 T m\(^{-1}\), 1 ms, and 2 ms, respectively. For simplicity, only single quantum coherence is considered here.

### 3.2.2 PGSE-WATERGATE

In 2002, Price et al. [96] developed one of the most popular water suppression PGSE
methods, PGSE-WATERGATE. As shown in Figure 3–7, in the PGSE-WATERGATE sequence, the solvent resonance will be destroyed by the same mechanism as WATERGATE. The PGSE-WATERGATE sequence was developed by integrating the solvent suppression and PGSE unit into a whole instead of connecting the solvent suppression unit to the PGSE unit and thus elegantly avoided the deleterious effects of radiation damping. In practice, the PGSE-WATERGATE sequence is extremely easy to set up: with the starting gradient strength (e.g., 0.05 T m\(^{-1}\)) being set high enough to kill the solvent resonance, the gradient strength is increased linearly to attenuate the resonances from molecules of interest. The development of the PGSE-WATERGATE sequence provides us a vital rule of developing new solvent suppression PGSE methods that the solvent suppression unit should be inside the PGSE unit.

![Diagram of PGSE-WATERGATE sequence](image)

**Figure 3–7** The PGSE-WATERGATE sequence [96]. The vertical bar grouping stands for a selective $\pi$ pulse, shaped gradients are used to minimize eddy current effects. For simplicity, only single quantum coherence is considered here.
3.2.3 Binomial-like Sequences

Based on the discussion above, the core parts of the WATERGATE and PGSE-WATERGATE sequence are the selective $\pi$ pulses, which determine the selectivity of the solvent suppression. Since being introduced by Hore, binomial sequences [97, 98], which have RF pulse durations in the ratio of binomial coefficients, have been widely used for selective excitation and inversion due to their good performance and ease of setup. The development of the binomial sequences are based on the inverse Fourier transform of [98]

$$\sin^n(\omega \tau/2)$$ (3.18)

or

$$\cos^n(\omega \tau/2)$$ (3.19)

which gives a wanted excitation/inversion spectrum, where $n$ is a positive integer. The inverse Fourier transform of $\sin^n(\omega \tau/2)$ (e.g., [98]),

$$\sum_{k=0}^{k=n} (-1)^k \binom{n}{k} \delta(t + [k - n/2] \tau),$$ (3.20)

is composed of $n + 1$ delta functions equally separated by $\tau$ with alternating signs and amplitudes given by the binomial coefficients $\binom{n}{k}$. This expression indicates the desired selective pulse sequences consist of groups of RF pulses with durations at the ratio of binomial coefficients separated by equal inter-pulse delays $\tau$, and the $(-1)^k$ indicates the phase of each RF pulse (i.e., $+1$ gives a phase of $0^\circ$ and $-1$ gives a phase of $180^\circ$). Similarly, the inverse Fourier transform of $\cos^n(\omega \tau/2)$ (e.g., [98]),
\[
\sum_{k=0}^{n} \binom{n}{k} \delta(t + [k - n/2] \tau),
\] (3.21)

give a family of selective pulse sequences analogous to the ones derived from Eq. (3.20) but with all the RF pulses in one sequence having the same phase.

To gain higher performance, binomial-like sequences (e.g., the 3-9-19 sequence [95] and W5 sequence [99], which have the same symmetric pulse arrangement as binomial sequences but pulse durations in arbitrary ratios, have been developed by optimizing pulse durations using numerical methods. A general binomial-like sequence can be written as [98]

\[
\alpha_i (\phi_i) - \tau - \alpha_2 (\phi_2) - \tau \cdots \alpha_n (\phi_n) - \tau - \alpha_{n+1} (\phi_{n+1}) \cdots \tau - \alpha_2 (\phi_{2n-1}) - \tau - \alpha_1 (\phi_{2n}),
\] (3.22)

where \( \alpha_i \) (i = 1, 2, ..., n) is the nutation angle of the RF pulse, \( \phi_i \) (i = 1, 2, ..., 2n) is the pulse phase, and \( \tau \) is the inter-pulse delay. Traditionally, the pulse phase is either 0° or 180°, for example, the 3-9-19-19-9-3 [95] and W5 [99] sequences can be written as

\[
20.77°(0°) - \tau - 62.31°(0°) - \tau - 131.54°(0°) - \tau - 131.54°(180°) - \tau - 62.31°(180°) - \tau - 20.77°(180°)
\] (3.23)

and

\[
7.8°(0°) - \tau - 18.5°(0°) - \tau - 37.2°(0°) - \tau - 70°(0°) - \tau - 134.2°(0°) - \tau - 7.8°(180°) - \tau - 18.5°(180°) - \tau - 37.2°(180°) - \tau - 70°(180°) - \tau - 134.2°(180°),
\] (3.24)

respectively.
3.3 MQ PGSE Experiments

It is often challenging to accurately determine the diffusion coefficients of macromolecules and macromolecular aggregates because of the relatively short transverse and longitudinal relaxation times and extremely slow diffusion ($D < 10^{-11}$ m$^2$ s$^{-1}$) of these particles. Typical high resolution probes have limited strength (e.g., $g_{\text{max}} = 0.6$ T m$^{-1}$) and thus normally can’t generate enough echo attenuation to precisely measure the slow diffusion of these particles within a relatively short diffusion time (e.g., 200 ms) which is limited by the relaxation times. For example, using the original Hahn spin-echo PGSE sequence with $\delta = 5$ ms, $\Delta = 200$ ms, and a maximum gradient strength of 0.6 T m$^{-1}$, a 70-80% attenuation (i.e., with 30%-20% of the original signal intensity remaining) can be achieved for a diffusing species with $D = 1 \times 10^{-11}$ m$^2$ s$^{-1}$, that is nothing smaller than this diffusion coefficient can be accurately determined. To deal with these problems, MQ PGSE experiments have been proposed. Up to now, there is no clear definition for MQ PGSE. Based on our knowledge, a MQ PGSE experiment should be a PGSE experiment in which more than one type of coherence state has been involved.

A good example of MQ PGSE sequences is the MAXY-D sequences [100, 101]. Compared with the normal single quantum PGSE experiments, the MAXY-D sequences provide higher signal attenuation with the same gradient durations and intensities by encoding the maximum quantum coherences, which have significantly higher $\gamma_{\text{eff}}$ values (e.g., for the quadruple quantum (QQ) coherence of $^8\text{H}_x^1\text{H}_y^1\text{H}_y^1\text{C}_x$, $\gamma_{\text{eff}} = 13 \gamma_{\text{H}}/4$) than the single quantum coherence (e.g., $\gamma_{\text{H}}$). These sequences are referred to as $\gamma_{\text{eff}}$– enhancement based PGSE sequences in this thesis.
The core of the MAXY-D sequences is \(90^\circ_x(I) - 1/(2J_{\nu_{C^1H}}) - 180^\circ_y(I) 90^\circ_x(S) - 1/(2J_{\nu_{C^1H}}) - 90^\circ_x(I)\) unit of the MAXY sequence [102], a modified combination of the conventional DEPT [103] and BIRD [104] sequences. The evolution of product operators for the \(90^\circ_x(I) - 1/(2J_{\nu_{C^1H}}) - 180^\circ_y(I) 90^\circ_x(S) - 1/(2J_{\nu_{C^1H}}) - 90^\circ_x(I)\) unit is [102]

\[
\begin{align*}
SI_n : I_z & \xrightarrow{90^\circ_x(I)} -I_y & & \xrightarrow{\pi/J_{\nu_{C^1H}}(2I_y S_y)} 2I_x S_y & & \xrightarrow{180^\circ_y(I) 90^\circ_y(S)} 2I_x S_y \\
XI_n : I_z & \xrightarrow{\pi/J_{\nu_{C^1H}}(2I_y S_y)} 2I_x S_y & & \xrightarrow{-I_y} -I_y & & \xrightarrow{90^\circ_x(I)} 2I_x S_y \\
-4I_x I_z S_x & \quad & 4I_x I_y S_y \\
-8I_x I_z I_z S_y & \quad & -8I_x I_y I_y S_y \\
-I_y & \quad & -I_z \\
\end{align*}
\]

(3.25)

here \(SI_n\) stands for \(^{13}\text{C}^1\text{H}, \)\(^{13}\text{C}^1\text{H}_2, \) and \(^{13}\text{C}^1\text{H}_3\) and \(XI_n\) stands for \(^{12}\text{C}^1\text{H}, \)\(^{12}\text{C}^1\text{H}_2, \) and \(^{12}\text{C}^1\text{H}_3\). As shown in Eq. (3.25), the \(90^\circ_x(I) - 1/(2J_{\nu_{C^1H}}) - 180^\circ_y(I) 90^\circ_x(S) - 1/(2J_{\nu_{C^1H}}) - 90^\circ_x(I)\) unit generates the maximum quantum coherences (i.e., double quantum (DQ) coherence for \(^{13}\text{C}^1\text{H}, \) TQ coherence for \(^{13}\text{CH}_2, \) and QQ coherence for \(^{13}\text{C}^1\text{H}_3\)), which are encoded in the MAXY-D experiments.
Chapter 4  Materials and Methods

4.1 Materials

$^2\text{H}_2\text{O}$ (99.9 atom %D) was purchased from ISOTEC, Matheson, Miamisburg, OH. 2 mM Glucose + 0.5 mM DSS + 2 mM NaN$_3$ in water (10:90, $^2\text{H}_2\text{O}:\text{H}_2\text{O}$) and 2 mM lysozyme in water (10:90, $^2\text{H}_2\text{O}:\text{H}_2\text{O}$) were both purchased as water suppression standard samples from Bruker, Karlsruhe, Germany. $[^{13}\text{C}]$-methanol was purchased as $^{13}\text{C}$ pulse calibration standard from Bruker, Karlsruhe, Germany. L-[3-$^{13}\text{C}$]-alanine was purchased from ISOTEC.

< 106 $\mu$m and 212-300 $\mu$m acid washed glass beads were both purchased from Sigma-Aldrich Inc., St. Louis, MO.

4.2 Computing

Simulation and data analysis were performed on a PC. Maple 11 (Maplesoft, Waterloo) was used for all NMR simulations. Origin 8 (OriginLab, Northampton, MA) was used for all diffusion data analysis.

4.3 NMR Techniques

4.3.1 $^1\text{H}$ NMR

$^1\text{H}$ NMR spectra were acquired on a Bruker Avance 400 spectrometer (Karlsruhe, Germany) at 400 MHz using a BBO probe and a Bruker Avance 500 spectrometer
(Karlsruhe, Germany) at 500 MHz using a BBI probe and a TXI probe. Typical acquisition parameters were: spectral width 7 kHz; digitized into 20 K data points; $\pi/2$ pulse length 8-14 $\mu$s.

$^1$H PGSE NMR spectra were acquired on a Bruker Avance 400 spectrometer at 400 MHz using a BBO probe and a Bruker Avance 500 spectrometer at 500 MHz using a BBI probe, a TXI probe, and a diffusion probe. Typical acquisition parameters for the BBO, BBI and TXI probes were: spectral width 7 kHz; digitized into 20 K data points; $\pi/2$ pulse length 8-14 $\mu$s. When diffusion experiments were performed on water saturated glass bead packs, typical acquisition parameters for the BBI and TXI probe were: spectral width 1 MHz; digitized into 5 K data points; $\pi/2$ pulse length 9.3 $\mu$s. Typical acquisition parameters for the diffusion probe were: spectral width 1 MHz; digitized into 18 K data points; $\pi/2$ pulse length 10.1 $\mu$s.

### 4.3.2 $^{13}$C NMR

$^{13}$C’s were excited on a Bruker Avance-500 spectrometer at 125.76 MHz with a $^{13}$C $\pi/2$ pulse length of 12.2 $\mu$s.

### 4.3.3 Temperature Calibration

Temperature calibration, when required, was performed using either an ethylene glycol or methanol sample [105, 106].

### 4.3.4 Relaxation Measurements

The longitudinal relaxation measurements were performed using the inversion-recovery pulse sequence (e.g., [32]). A delay $\geq 5 T_1$ was allowed between transients [107]. At least eight transients were averaged for each spectrum.
4.4 NMR Sample Preparation

Volumes of 0.6 ml of each sample were dispensed into 5-mm NMR tubes (Wilmad, Buena, NJ). Volumes of 0.3 ml of each sample were dispensed into magnetic susceptibility-matched (to \(^2\text{H}_2\text{O}\)) NMR tubes (BMS-003, Shigemi, Tokyo) and glass bead packs in 5-mm NMR tubes (Wilmad, Buena, NJ).
Chapter 5  MAG-PGSTE: A New STE-Based
PGSE Sequence for Diffusion Measurements in
Magnetically Inhomogeneous Samples

5.1 Preamble
With short exposure (i.e., encoding periods (\(\tau_e\)) to background gradients and \(T_2\) relaxation, STE \((\pi/2 - \tau_e - \pi/2 - \tau_s - \pi/2 - \tau_e - \text{Echo})\)-based PGSE sequences outperform Hahn spin-echo based PGSE sequences in the presence of background gradients. By replacing the initial symmetric bipolar gradient pairs by asymmetric bipolar gradient pairs, Sun et al. [89, 108] and Galvosas et al. [109, 110] independently modified the Cotts 13-interval sequence [72] into a series of STE-based PGSE sequences (i.e., MAGSTE (Figure 5–1A) or MPFG sequences) containing asymmetric gradient pairs with the amplitudes of the gradient pulses set at “magic” ratios which result in a zero cross-term in the presence of non-constant background gradients and these sequences can suppress the effects of non-constant background gradients in one transient. For the 13-interval sequence [72], the cross-term between the applied and background gradients generated in the first encoding period is cancelled by the cross-term generated in the second encoding period with appropriate parameter settings (i.e., \(E_{\text{cross}} \neq 0\) at the end of the first encoding period and \(E_{\text{cross}} = 0\) at the end of the second encoding period; \(E_{\text{cross}}\) is the cross-term based
spin-echo attenuation.) [70, 72]; while for the MAGSTE or MPFG sequence, the cross-terms generated in the two encoding periods are suppressed independently (i.e., $E_{\text{cross}} = 0$ at the end of the first or second encoding period) [89, 110]. In this study, only the MAGSTE sequence has been studied.

Figure 5–1 (A) The MAGSTE sequence [89]. The phase cycle for the pulse sequences is $\phi_1 = x; \phi_2 = x, -x, x, -x, y, -y, y, -y; \phi_3 = x, x, -x, y, y, -y, -y; \phi_4 = y; \phi_5 = y, \phi_r$ (receiver phase) = $x, -x, -x, x$. (B) The MAG-PGSTE sequence. The phase cycle for the pulse sequences is $\phi_1 = x; \phi_2 = x, -x, x, -x, y, -y, y, -y; \phi_3 = x, x, -x, x, y, y, -y, -y; \phi_4 = y; \phi_5 = y, y, -y, -y, -y, -x, x, x; \phi_r$ (receiver phase) = $x, -x, -x, x$. The narrow bars represent $\pi/2$ and $\pi$ RF pulses, $g_{01}$ and $g_{02}$ are background gradients, $g_1$, $g_2$ and crusher are rectangular gradient pulses with different amplitudes. The variable gradient fill for $g_{01}$ and $g_{02}$ signifies that the background gradients may fluctuate during encoding periods.
It has been shown that the 17-interval sequence outperforms the 13-interval sequence in the suppression of the $g_0$-only-based spin-echo attenuation ($E_{g_0}$), which becomes very significant in the presence of high background gradients [72]. Therefore, a new STE-based PGSE sequence, MAG-PGSTE (Figure 5–1B), which can suppress the effects of non-constant background gradients with higher gradient encoding efficiency [83] and signal-to-noise (S/N) ratio, has been developed by replacing the symmetric bipolar gradient pairs in the 17-interval sequence by asymmetric bipolar gradient pairs with the gradient amplitudes set at the “magic” ratio [89, 110]. The utility of the new sequence is demonstrated using two samples containing glass bead packs (NB with diameters < 106 μm and 212 – 300 μm) filled with CuSO$_4$-doped water. The performance of the new sequence is contrasted with the MAGSTE and Cotts 13-interval sequence.

5.2 Preparation of Glass Bead Samples and Diffusion Measurements

Micro-spherical acid-washed glass beads with sizes of < 106 μm and 212 - 300 μm were transferred into two 5 mm NMR tubes and then saturated with 0.01 M CuSO$_4$·5H$_2$O solution. Another 5 mm NMR tube containing 0.01 M CuSO$_4$·5H$_2$O solution was used as a reference sample. The diffusion coefficient of water in the reference sample was found to be $2.33 \pm 0.01 \times 10^{-9}$ m$^2$ s$^{-1}$ at 25 ºC using the original Hahn spin-echo PGSE sequence.

All measurements were conducted on a Bruker 500 MHz Avance NMR spectrometer using a high-resolution gradient NMR probe (i.e., Bruker BBI probe) and a high-gradient NMR probe (i.e., Bruker Diff30 probe) at 25 ºC. Diffusion was measured using the MAG-PGSTE, MAGSTE [89], and Cotts 13-interval sequences [72] with half-sine shaped gradients and recycle delay = 5 ms (NB $> 5 \times T_1$). For the
high resolution gradient probe, diffusion experiments were performed with $\delta = 3$ ms, $\tau_e = 10$ ms, $\tau_2 = 3.05, 5.05, 7.05, 10.05, 15.05, 22.05, 31.05, 46.05, 63.05, 91.05, 127.05, 255.05, 511.05$ ms (for the MAG-PGSTE and Cotts 13-interval sequence) or $7.05, 10.05, 15.05, 22.05, 31.05, 46.05, 63.05, 91.05, 127.05, 255.05, 511.05$ ms (for the MAGSTE sequence), and each diffusion measurement involved 21 $z$-gradient strengths (e.g., $g_1$ for the MAG-PGSTE sequence and $g_2$ for the MAGSTE sequence) that varied linearly from 0.005 T m$^{-1}$ to the value which gave an attenuation of 80-90% (i.e., 20%-10% of the original intensity remaining); for the high gradient probe, diffusion experiments were performed with $\delta = 0.5$ ms, $\tau_e = 3.5$ and 10 ms, $\tau_2 = 3.18, 9.18, 17.18, 33.18, 65.18, 129.18, 257.18, 513.18$ ms for the MAG-PGSTE and Cotts 13-interval sequence and $9.18, 17.18, 33.18, 65.18, 129.18, 257.18, 513.18$ ms for the MAGSTE sequence, and each diffusion measurement involved 21 $z$-gradient strengths that varied linearly from 0.036 T m$^{-1}$ to the value which gave an attenuation of 80-90%.

The time-dependent diffusion coefficient was determined from fitting the single-exponential equation $y = Me^{-b_0 D(\Delta)}$ ($M$ is a constant, $b_0$ and $D(\Delta)$ are defined in Eqs. (4.10) and (4.14), respectively) to the echo attenuation profiles.

## 5.3 Results and Discussion

To differentiate the effects of applied and background gradients, the total spin-echo attenuation in the presence of background gradients can be rewritten as

$$
\frac{S_{t=acq}}{S_{t=0}} = E_g E_{cross} E_{ge}
$$

(4.1)

where, $E_g$ is the attenuation caused by the applied gradients only.
**MAG-PGSTE sequence with rectangular pulsed gradients**

For the MAGSTE sequence [89] with rectangular gradients (Figure 5–1B), $E_g$, $E_{\text{cross}}$ and $E_{\text{bg}}$ can be calculated by evaluating Eq. (2.32) over each period of the pulse sequence to be (neglecting relaxation processes)

\[
E_g = \exp \left\{ -\gamma^2 D \delta^2 \left[ \left( \Delta + 2\tau_1 + \delta_2 - \delta_1 - \frac{\delta}{3} \right)(g_1 - g_2)^2 
+ \left( 8\tau_1 + 4(\delta_2 - \delta_1) - \frac{2\delta}{3} \right)(g_1 - g_2)g_2 \right. 
+ \left. \left( 4\tau_1 + 2(\delta_2 - \delta_1) - \frac{2\delta}{3} \right)g_2^2 \right] \right\}.
\]

(4.2)

\[
E_{\text{cross}} = \exp \left\{ \left( -\gamma^2 D \delta g_2 / 3 \right)[(5\delta^2 - 9\delta_1 \delta_2 - 3\delta_1^2 - 12\delta_1 \delta_2 - 12\delta_2 \delta_1 - 6\delta_2^2) \eta_{\text{MAGSTE}}
+ 3\delta_1 \delta + 3\delta_1^2 + \delta^2) g_{01}
+ \left( (5\delta^2 - 9\delta_1 \delta - 3\delta_1^2 - 12\delta_1 \delta_2 - 12\delta_2 \delta - 6\delta_2^2) \eta_{\text{MAGSTE}}
+ 3\delta_1 \delta + 3\delta_1^2 + \delta^2) g_{02} \right] \right\},
\]

(4.3)

and

\[
E_{\text{bg}} = \exp \left\{ -\frac{16}{3} \gamma^2 D \tau_1^3 \left( g_{01}^2 + g_{02}^2 \right) \right\}.
\]

(4.4)

where $g_1$, $g_2$, $g_{01}$, $g_{02}$, and the timing parameters are defined in Figure 5–1A, and $\eta_{\text{MAGSTE}} = g_1 / g_2$. As shown in Eq. (4.3), there are two terms, the $g_{01}$-term and the $g_{02}$-term, in $E_{\text{cross}}$. Because $g_{01} \neq g_{02}$ and neither of them is known, both of the two terms must be equal to zero so that a zero cross-term can be achieved. Therefore, by solving $g_{01}$-term = 0 and $g_{02}$-term = 0 for $\eta_{\text{MAGSTE}}$, the magic gradient amplitude ratio for suppressing the cross-terms between the applied and background gradients is
determined to be [89]

$$\eta_{\text{MAGSTE}} = \frac{3\delta_1\delta + 3\delta_1^2 + \delta^2}{5\delta^2 + 9\delta_1\delta + 3\delta_2^2 + 12\delta_1\delta_2 + 12\delta_2\delta + 6\delta^2}. \quad (4.5)$$

Although the MAGSTE sequence can suppress $E_{\text{cross}}$, it only has one $\pi$ pulse in each encoding period and thus low efficiency in the suppression of $E_{g_6}$; $E_{g_6}$ becomes significant when $g_0$ is large. The FWHM’s of the spectra of the 212 - 300 $\mu$m and < 106 $\mu$m glass bead samples were 2181 and 2483 Hz, respectively. The background gradients in the 212 - 300 $\mu$m and < 106 $\mu$m glass bead samples have been estimated to be 0.2 T m$^{-1}$ and 1.1 T m$^{-1}$ by the use of Eq. (3.15), respectively, which are comparable to the maximum pulsed gradient strength of commercial high-resolution gradient NMR probes (e.g., 0.6 T m$^{-1}$) and thus can cause a poor S/N ratio. Therefore, more $\pi$ pulses are needed in the encoding periods to achieve more efficient suppression of $E_{g_6}$. The MAG-PGSTE sequence has been developed for the suppression of both $E_{\text{cross}}$ and $E_{g_6}$.

For the newly developed MAG-PGSTE sequence with rectangular pulsed gradients, $E_g$, $E_{\text{cross}}$ and $E_{g_6}$ can be calculated by evaluating Eq. (2.32) over each period of the pulse sequence to be (neglecting relaxation processes)

$$E_g = \exp\left\{-\gamma^2D\delta^2\left[\left(\Delta + 2\tau_1 + \delta_3 - \delta_1 - \frac{2\delta}{3}\right)(g_1 - g_2)\right]^2ight. \right.$$

$$+ \left.\left(8\tau_1 + 4(\delta_3 - \delta_1) - \frac{2\delta}{3}\right)g_1 g_2 \right), \quad (4.6)$$

$$+ \left.\left(4\tau_1 + 2(\delta_3 - \delta_1) - \frac{2\delta}{3}\right)g_2^2 \right\}$$
\[ E_{\text{cross}} = \exp\left(-\gamma^2 D \delta g_1 / 3\right) \left[ (3 \tau_1^2 + 6 \tau_1 \delta_3 + 3 \tau_1 \delta - 3 \delta_3 \delta - \delta^2 - 3 \delta_3^2)\eta_{\text{MAG-PGSTE}} \right. \\
\left. -3 \tau_1^2 + 6 \tau_1 \delta_3 + 3 \tau_1 \delta - 3 \delta_3 \delta - \delta^2 - 3 \delta_3^2 \right] g_{01} \\
+ \left( (-3 \tau_1^2 - 6 \tau_1 \delta_1 - 3 \tau_1 \delta + 3 \delta_1 \delta + \delta^2 + 3 \delta_1^2)\eta_{\text{MAG-PGSTE}} \right. \\
\left. +3 \tau_1^2 - 6 \tau_1 \delta_1 - 3 \tau_1 \delta + 3 \delta_1 \delta + \delta^2 + 3 \delta_1^2 \right] g_{02} \right), \quad (4.7) \\
\]

and

\[ E_{g_0} = \exp\left(-2 \gamma^2 D \tau_1^3 \left( g_{01}^2 + g_{02}^2 \right) \right) \]

where \( g_1, g_2, g_{01}, g_{02} \), and the timing parameters are defined in Figure 5–1B, and \( \eta_{\text{MAG-PGSTE}} = g_2 / g_1 \). By solving \( g_{01} \)-term = 0 and \( g_{02} \)-term = 0 for \( \eta_{\text{MAG-PGSTE}} \) when \( \delta_1 = \delta_3 \) or \( \delta_2 = \delta_3 \), the magic gradient amplitude ratio for suppressing the cross-terms between the applied and background gradients is determined to be

\[ \eta_{\text{MAG-PGSTE}} = \frac{3 \tau_1^2 - \left( 6 \tau_1 \delta_1 + 3 \tau_1 \delta - 3 \delta_1 \delta - 3 \delta_1^2 - \delta^2 \right) \left( 3 \tau_1^2 + \left( 6 \tau_1 \delta_1 + 3 \tau_1 \delta - 3 \delta_1 \delta - 3 \delta_1^2 - \delta^2 \right) \right)}{3 \tau_1^2 + \left( 6 \tau_1 \delta_1 + 3 \tau_1 \delta - 3 \delta_1 \delta - 3 \delta_1^2 - \delta^2 \right)} \]. \quad (4.9) \\

For the MAGSTE sequence developed by Sun et al. [89], the cross-term caused by non-constant background gradients can be suppressed only when the bipolar gradients are symmetrically placed around \( \pi \) pulses. However, for the newly developed MAG-PGSTE sequence, a unique magic ratio (i.e., \( \eta_{\text{MAG-PGSTE}} = g_2 / g_1 \)) has been obtained for the suppression of the cross-term when \( \delta_1 = \delta_3 \) or \( \delta_2 = \delta_3 \). In particular, when \( \delta_1 = \delta_3 \), both \( \delta_1 \) and \( \delta_3 \) can be set as small as possible to minimize the eddy current effects [46] caused by the application of pulsed gradients. The MPFG sequence also has this property [110].

Comparing the MAGSTE and MAG-PGSTE sequences, Eqs. (4.2) and (4.6)
give the same $E_g$. However, comparing Eqs. (4.4) and (4.8), the MAG-PGSTE sequence gives a smaller $E_{g_0}$ than the MAGSTE sequence, indicating that the spin-echo signal is less attenuated by $E_{g_0}$ in an MAG-PGSTE experiment than in an MAGSTE experiment and thus the MAG-PGSTE sequence provides a better S/N ratio. As shown in Figure 5-2, for the sample containing CuSO$_4$-doped H$_2$O and < 106 μm glass beads, the MAG-PGSTE spectra have a much better S/N ratio than the MAGSTE spectra.

Figure 5-2 A series of 500 MHz $^1$H (A) MAG-PGSTE and (B) MAGSTE spectra on the sample containing CuSO$_4$-doped water and < 106 μm glass beads. For both sequences, $\delta = 3$ ms, $\tau_e = 10$ ms, $\Delta = 41$ ms, receiver gain = 128 (MAG-PGSTE) and 812 (MAGSTE). As can be clearly seen the MAG-PGSTE spectra have much higher S/N.
Based on the simulations, the MAG-PGSTE sequence has a magic ratio ($\eta_{\text{MAG-PGSTE}}$) smaller than the magic ratio for the MAGSTE sequence ($\eta_{\text{MAGSTE}}$), and thus giving higher applied gradient encoding efficiency [83]. As shown in Figure 5–3, when $\delta_2 = 0.2$ ms with $\delta_1$ ranging from 0 to 0.5 ms and $\delta$ ranging from 0.8 to 4 ms, $\eta_{\text{MAG-PGSTE}}$ ranges from 0.07 to 0.18; while, in Figure 5–4, when $\delta_2 = 0.2$ ms with $\delta_1$ ranging from 0.2 to 0.5 ms and $\delta$ ranging from 0.8 to 4 ms, $\eta_{\text{MAGSTE}}$ ranges from 0.17
MAG-PGSTE sequence with half-sine gradients

Shaped pulsed gradients have been widely used in PGSE experiments to avoid the deleterious effects of eddy currents generated by switching on/off gradients [111]. When using high power gradients (e.g., \( g_{\text{max}} = 17.9 \text{ T m}^{-1} \)), the shaped gradients become necessary because their slow rising and falling result in less eddy current generation. Therefore, the MAG-PGSTE sequence with half-sine pulsed gradients was developed (Figure 5–5).

\[
E_g = \exp \left\{ -\frac{1}{\pi^2} r^2 D^2 \delta^2 \left[ (4\Delta + 8\tau_1 - \delta + 4\delta_3 - 4\delta_1)(g_1 - g_2)^2 + (32\tau_1 - 2\delta + 16\delta_3 - 16\delta_1)(g_1 - g_2)g_2 + (16\tau_1 - 2\delta - 8\delta_3 - 8\delta_1)g_2^2 \right] \right\} = \exp(-b_g D),
\]

Figure 5–5 The MAG-PGSTE sequence with half-sine gradients.
\[ E_{\text{cross}} = \exp \left\{- \frac{1}{\pi} \gamma^2 \delta g_1 D \left[ g_{01} \left( \eta_{\text{MAG-PGSTE}} \left( 4 \delta_1^2 - \pi^2 \delta_1^2 + 2 \pi^2 \tau_1^2 + 4 \pi^2 \tau_1 \delta_1 + 2 \pi^2 \tau_1 \delta_2 - 2 \pi^2 \tau_1 \delta_3 \right) \right. \right. \]
\[ -2 \pi^2 \delta_1^2 + 2 \pi^2 \tau_1 \delta_2 - 2 \pi^2 \delta_3 \left. \right) \right. \]
\[ - \left( \pi^2 \delta_1^2 + 2 \pi^2 \delta_2 - 2 \pi^2 \tau_1 \delta - 4 \delta_2 \right. \]
\[ + 2 \pi^2 \tau_1^2 - 4 \pi^2 \tau_1 \delta_1 + 2 \pi^2 \delta_2 \left. \right] \right \}
\[ + g_{02} \left( \eta_{\text{MAG-PGSTE}} \left( -4 \delta_1^2 + \pi^2 \delta_1^2 - 2 \pi^2 \tau_1^2 - 4 \pi^2 \tau_1 \delta_1 \right. \right. \]
\[ + 2 \pi^2 \delta_1^2 - 2 \pi^2 \tau_1 \delta + 2 \pi^2 \delta_2 \left. \right) \right. \]
\[ + \left( \pi^2 \delta_1^2 + 2 \pi^2 \delta_2 - 2 \pi^2 \tau_1 \delta - 4 \delta_2 \right. \]
\[ + 2 \pi^2 \tau_1^2 - 4 \pi^2 \tau_1 \delta_1 + 2 \pi^2 \delta_2 \right \} \right] \right)
\[ = \exp (-b_{\text{cross}} D), \]

and

\[ E_{g_s} = \exp \left[ -2 \gamma^2 \tau_1^3 D (g_{01}^2 + g_{02}^2) \right] \]
\[ = \exp (-b_{g_s} D), \] (4.12)

respectively.

By solving \( g_{01} \)-term = 0 and \( g_{02} \)-term = 0 for \( \eta_{\text{MAG-PGSTE}} \) when \( \delta_1 = \delta_3 \) or \( \delta_2 = \delta_3 \), the magic gradient amplitude ratio for suppressing the cross-terms between the applied and background gradients is determined to be

\[ \eta_{\text{MAG-PGSTE}} = \frac{2 \tau_1^2 - \left( 2 \delta \tau_1 + 4 \delta_1 \tau_1 - \delta_1^2 - 2 \delta \delta_1 - 2 \delta_2 + 4 \delta_2^2 / \pi^2 \right)}{2 \tau_1^2 + \left( 2 \delta \tau_1 + 4 \delta_1 \tau_1 - \delta_1^2 - 2 \delta \delta_1 - 2 \delta_2^2 + 4 \delta_2^2 / \pi^2 \right)}, \] (4.13)
Diffusion measurements on glass bead samples (using high resolution probe)

In general, the strong background gradients in porous media are of interest. Inside porous media, the measured diffusion coefficients become time-dependent due to the existence of geometrical restrictions and this time-dependence carries structural information of the porous media. At short diffusion times, the time-dependent diffusion coefficient is defined as [24, 25]

\[
D(\Delta) = D_0 \left[ 1 - \frac{4}{9\sqrt{\pi}} \frac{S}{V} \sqrt{D_0 \Delta} + O(D_0 \Delta) \right],
\]

(4.14)

where \(D_0\) is the free diffusion coefficient, \(S/V\) is the surface-to-volume ratio of the pores.

Including the time dependence of measured diffusion coefficients, Eqs. (4.10)-(4.12) can be rewritten as [23, 24, 77]

\[
E_g = \exp \left( -b_g D(\Delta) \right),
\]

(4.15)

\[
E_{\text{cross}} = \exp \left( -b_{\text{cross}} D(\Delta) \right),
\]

(4.16)

and

\[
E_{g_0} = \exp \left( -b_{g_0} D(\Delta) \right).
\]

(4.17)

To assure the validity of Eqs. (4.15)-(4.17) in the data analysis, the maximum echo attenuation was controlled to be 80% (i.e., 20% of the original intensity remaining) in all data analysis and the condition, namely \(qL >> 1\) (\(q = (2\pi)^{-1} g g_0 \delta\) and \(L\)
is the length of the sample), was also fulfilled by setting the minimum gradient strength to 0.005 T m\(^{-1}\) in all the experiments [78].

![Figure 5–6 A plot of \(D(\Delta)\) vs. \(\Delta\) for the diffusion measurements of < 106 \(\mu\)m and 212-300 \(\mu\)m glass bead samples are shown in solid and open symbols, respectively. The data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequence are indicated by squares, circles, and triangles, respectively.](image)

The utility of the MAG-PGSTE sequence has been demonstrated on glass bead packs filled with CuSO\(_4\)-doped water. To show the dependence of \(D(\Delta)\) on \(\Delta\), \(D(\Delta)\) was plotted against \(\Delta\) (Figure 5–6). As shown in Figure 5–6, all diffusion measurements were lower than the diffusion coefficient of water in the reference sample (i.e., \(2.33 \pm 0.01 \times 10^{-9} \text{ m}^2\text{s}^{-1}\)) due to the effects of highly intense and inhomogeneous background gradients [43, 85, 86, 89, 112], which is analogous to the apparent slowing down of diffusion in pores with relaxing boundaries [113]. The measured diffusion coefficients of water from the < 106 \(\mu\)m glass bead sample were significantly lower than that from the 212-300 \(\mu\)m due to higher intensity and inhomogeneity of background gradients. For the < 106 \(\mu\)m glass bead sample, the MAG-PGSTE sequence consistently gave higher diffusion measurements owing to its
high efficiency in the suppression of both $E_{\text{cross}}$ and $E_{g0}$ except that the MAGSTE sequence gave the highest diffusion measurement when $\Delta = 17.05$ ms. For the 212-300 $\mu$m glass bead sample, the MAGSTE sequence consistently gave higher diffusion measurements at short diffusion times (i.e., $\leq 100$ ms) due to its higher efficiency in the suppression of $E_{\text{cross}}$ while, at long diffusion times, the MAGSTE diffusion measurements overlapped with the MAG-PGSTE and Cotts-13 interval diffusion measurements.

Table 5-1 Bead diameters and $S/V$ ratios of the glass bead samples determined from data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequence. $S/V$ ratios were obtained by fitting Eq. (4.14) to the diffusion data at short diffusion times (i.e., $\leq 100$ ms) and the measured bead diameters were calculated by the use of $d = 6 \left( \frac{1}{\phi} - 1 \right) / S/V$ [23] with a porosity of $\phi = 0.38$ (e.g., [23]).

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Measured diameter ($\mu$m)</th>
<th>$S/V$ ($\mu$m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAG-PGSTE</td>
<td>&lt; 106 $\mu$m beads</td>
<td>89</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>212-300 $\mu$m beads</td>
<td>245</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>MAGSTE</td>
<td>&lt; 106 $\mu$m beads</td>
<td>61</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>212-300 $\mu$m beads</td>
<td>245</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Cotts 13-interval</td>
<td>&lt; 106 $\mu$m beads</td>
<td>122</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>212-300 $\mu$m beads</td>
<td>326</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

To test the sphere size characterization capability of the MAG-PGSTE sequence, Eq. (4.14) was fitted to the diffusion measurements at short diffusion times (i.e., $\Delta \leq 100$ ms), and the measured bead diameters and $S/V$ ratios are shown in Table 5-1. The measured bead diameters using the MAG-PGSTE and MAGSTE sequence matched the nominal bead diameters very well. Although the Cotts 13-interval
sequence can differentiate between the two glass bead samples, it over-estimated the bead diameters for both glass bead samples.

**Diffusion measurements on glass bead samples (using high gradient probe)**

High power gradient pulses with durations of 0.5 ms were used in the experiments to minimize the encoding periods and thus minimize the effects of the fluctuation of background gradients. However, the use of high power gradients generates significant eddy currents which can deleteriously affect the application RF pulses and signal acquisition, and the delay after each gradient has to be set long enough to let the eddy currents die off before the application of RF pulses or acquisition. Therefore, it is necessary to find the best trade-off between minimizing encoding periods and suppressing the effects of eddy currents. The gradient-excitation-acquisition sequence (i.e., \( g \text{ – eddy current delay} \text{ – } \pi/2 \text{ – } \text{Acq} \)) has been used to determine eddy current delays. For a \( 12.6 \text{ T m}^{-1} \) rectangular gradient, the minimum eddy current delay has been found to be 0.5 ms. In following experiments, all eddy current delays are set to 0.5 ms, which is five times larger than the commonly used eddy current delays for a high resolution probe with a maximum gradient strength of \( 0.6 \text{ T m}^{-1} \) (e.g., 100 \( \mu \text{s} \)).

As was done for the experiments using the high resolution probe, the maximum echo attenuation was controlled to be 80\% (i.e., 20\% of the original intensity remaining) in all data analysis and the condition, namely \( qL \gg 1 \) (\( q = (2\pi)^{-1} \gamma g \delta \) and \( L \) is the length of the sample), was also fulfilled by setting the minimum gradient strength to \( 0.036 \text{ T m}^{-1} \)[78].
Figure 5–7 Representative plots of diffusion attenuation of the water resonance in glass beads samples with mean sizes of $< 106 \, \mu m$ at encoding times of 3.5 ms ($\ddagger$, $\slash$, and : ) and 10 ms ($\ddagger$, $0$, and ;) and $\tau_2$ of 9.18 (A), 17.18 (B), 65.18 (C), and 257.18 (D) ms. The data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequence are indicated by squares, circles, and triangles, respectively.

To show the dependence of echo attenuating behaviour on diffusion time, the logarithm of echo attenuations are shown in Figure 5–7 and Figure 5–8. For the $< 106 \, \mu m$ glass bead sample (Figure 5–7), when $\tau_2$ was set to 9 or 17 ms, both MAG-PGSTE and MAGSTE sequences gave the faster attenuations and thus the better diffusion measurements than the Cotts 13-interval sequence; at longer $\tau_2$ (i.e., 65 ms), the MAG-PGSTE sequence gave faster attenuation than the Cotts 13-interval sequence while the MAGSTE data started to overlap with the Cotts 13-interval data; when $\tau_2$ was set to 257 ms, the MAG-PGSTE sequence still gave faster attenuation...
than the Cotts 13-interval sequence while the MAGSTE data completely overlapped with the Cotts 13-interval data. More interestingly, when a longer encoding time (i.e., 10 ms) was used (shown by open symbols), the echo attenuations of the MAGSTE and Cotts 13-interval sequences were significantly deleteriously affected due to the signal loss caused by large $g_0^2$ term, while the echo attenuations of the MAG-PGSTE sequence were not influenced much due to the higher $g_0^2$ term suppression efficiency.

Figure 5–8 Representative plots of diffusion attenuation from glass beads samples with mean sizes of 212 - 300 μm at encoding times of 3.5 ms (§, /, , ) and 10 ms (%, 0, ;) and $\tau_2$ of 9.18 (A), 17.18 (B), 65.18 (C), and 257.18 (D) ms. The data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequence are indicated by squares, circles, and triangles, respectively.

For the 212 - 300 μm glass bead sample (Figure 5–8), when $\tau_2$ was set to 9 or 17 ms, the MAGSTE sequence gave slightly faster attenuation than the other two sequences; while at larger $\tau_2$ (i.e., 65 ms and 257 ms) the data from three sequences
completely overlapped. No deleterious effects as observed for the < 106 μm glass bead sample were observed for the 212 – 300 μm glass bead sample by using a longer encoding time (i.e., 10 ms), indicating the 212 – 300 μm glass bead sample is much less inhomogeneous than the < 106 μm glass bead sample.

In summary, the MAG-PGSTE sequence outperformed the MAGSTE and Cotts 13-interval sequence in the diffusion measurement on a highly magnetically inhomogeneous sample (i.e., the < 106 μm glass bead sample) while all three sequences gave similar performance on a sample with medium magnetic inhomogeneity (i.e., the 212 - 300 μm glass bead sample).

Figure 5–9 The measured diffusion coefficients as a function of the diffusion time for the < 106 μm (§, /, and :) and 212 - 300 μm (% , 0 , and ;) with an encoding time of 3.5 ms. The data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequence are indicated by squares, circles, and triangles, respectively.

The $D(\Delta)$’s of water in the glass bead samples were plotted against diffusion time $\Delta$ (Figure 5–9 and Figure 5–10). All the diffusion measurements for the glass bead samples were smaller than the diffusion coefficient of water in the reference
sample (i.e., $2.33 \pm 10^{-9} \, \text{m}^2 \, \text{s}^{-1}$), indicating that none of the three sequences can fully suppress rapidly varying background gradients, which may be very pertinent in systems with microscopic heterogeneities, where spin displacement during the encoding periods can be comparable to the length scale of the local structure. All diffusion measurements for the glass bead samples decreased over $\Delta$, which can be attributed to restricted diffusion when the MSD was comparable to the length scale of the diffusion barriers, causing the measured diffusion coefficients at longer diffusion times to plateau [114].

![Figure 5–10](image-url)

**Figure 5–10** The measured diffusion coefficients as a function of the diffusion time for the $< 106 \, \mu\text{m}$ ($\$, $\slash$, and $:\$) and $212 - 300 \, \mu\text{m}$ ($\%$, $0$, and $;$) with an encoding time of 10 ms. The data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequence are indicated by squares, circles, and triangles respectively.

When the encoding time was set to 3.5 ms (Figure 5–9), diffusion coefficient plateaued near 300 ms for the $< 106 \, \mu\text{m}$ glass bead sample, while the diffusion coefficient continued to decrease for the $212 - 300 \, \mu\text{m}$ glass bead sample when $\Delta > 400$ ms, owing to its larger pore size [114].

It was assumed that the background gradient was constant during each encoding period if these encoding periods are short enough to avoid the diffusion of
spins through the inhomogeneity of background gradients. Experiments were conducted with an extended encoding period of 10 ms. As shown in Figure 5–10, the diffusion measurements for the 212 - 300 μm glass bead sample were not affected by extending the encoding period due to smaller magnetic inhomogeneity in the sample, while the diffusion measurements for the < 106 μm glass bead sample dropped down significantly.

As shown in Figure 5–9, the MAG-PGSTE, MAGSTE, and Cotts 13-interval diffusion measurements for the 212 - 300 μm glass bead sample tended to converge at long diffusion times (i.e., Δ > 260 ms). In typical diffusion-diffraction experiments, in which structural information are obtained by observing restricted diffusion, diffusion times are set extremely long (e.g., a few seconds) so that most diffusing spins can ‘bump’ into the boundaries; therefore, the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequences will give similar results in diffusion-diffraction experiments on samples with medium magnetic inhomogeneity (i.e., medium pore sizes). However, it can also be seen in Figure 5–9 that for the < 106 μm glass bead sample the MAG-PGSTE diffusion measurements were consistently higher than other diffusion measurements at long diffusion times, indicating that the MAG-PGSTE sequence can provide more accurate diffusion-diffraction experiments on highly magnetically inhomogeneous samples (i.e., samples with small pore sizes).

The normalized diffusion measurements (i.e., $D_{\text{Cotts}}/D_{\text{MAG-PGSTE}}$, $D_{\text{Cotts}}/D_{\text{MAG-PGSTE}}$) are plotted as functions of Δ in Figure 5–11 and Figure 5–12. At short diffusion times, the normalized diffusion measurements were less than 1 due to the incomplete suppression of the heterogeneous background gradients. When the diffusion time was increased, the normalized diffusion measurements for the 212 - 300 μm glass bead sample plateaued at a value close to 1 due to the involvement of restricted diffusion at
long diffusion times; the normalized diffusion measurements for the < 106 μm glass bead sample didn’t plateau at long diffusion times.

Figure 5–11 The normalized diffusion coefficients (i.e., $\frac{D_{\text{Cotts}}}{D_{\text{MAG-PGSTE}}}$ (squares) and $\frac{D_{\text{Cotts}}}{D_{\text{MAGSTE}}}$ (circles)) for the encoding time of 3.5 ms from the < 106 μm ($\text{§}$ and $\text{/}$) and 212 - 300 μm ($\text{¥}$ and $\text{0}$) glass bead samples.

Figure 5–12 The normalized diffusion coefficients (i.e., $\frac{D_{\text{Cotts}}}{D_{\text{MAG-PGSTE}}}$ (squares) and $\frac{D_{\text{Cotts}}}{D_{\text{MAGSTE}}}$ (circles)) for the encoding time of 10 ms from the < 106 μm ($\text{§}$ and $\text{/}$) and 212 - 300 μm ($\text{¥}$ and $\text{0}$) glass bead samples.
Table 5-2 Bead diameters and $S/V$ ratios of the glass bead samples determined from the data obtained with the MAG-PGSTE, MAGSTE, and Cotts 13-interval sequences with encoding time = 3.5 ms. $S/V$ ratios were obtained by fitting Eq. (4.14) to the diffusion data at short diffusion times (i.e., $\leq 100$ ms) and the measured bead diameters are calculated by the use of $d = 6 \left( \frac{1}{\phi} - 1 \right) / S/V$ [23] with a porosity of $\phi = 0.38$ (e.g., [23]).

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Measured diameter</th>
<th>$S/V$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(µm)</td>
<td>(µm$^{-1}$)</td>
</tr>
<tr>
<td>MAG-PGSTE</td>
<td>&lt; 106 µm beads</td>
<td>109</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>212-300 µm beads</td>
<td>326</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>MAGSTE</td>
<td>&lt; 106 µm beads</td>
<td>98</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>212-300 µm beads</td>
<td>326</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Cotts 13-interval</td>
<td>&lt; 106 µm beads</td>
<td>109</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>212-300 µm beads</td>
<td>489</td>
<td>0.02 ± 0.01</td>
</tr>
</tbody>
</table>

Again, to test the sphere size characterization capability of the MAG-PGSTE sequence, Eq. (4.14) was fitted to the diffusion measurements at short diffusion times (i.e., $\Delta \leq 100$ ms), and the measured bead diameters and $S/V$ ratios are shown in Table 5-2.

For the < 106 µm glass bead sample, three sequences gave similar bead size and $S/V$ measurements; while for the 212 - 300 µm glass bead sample, all the three sequences overestimated the bead sizes. Comparing the results in Table 5-1 and Table 5-2, it is quite clear that using the high resolution probe provided a better sphere size characterization.
Chapter 6  New Solvent Suppression Methods for PGSE Experiments

6.1 Preamble

Solvent suppression in PGSE experiments has long been of interest. However, only a small number of solvent suppression techniques can achieve high suppression efficiency in combination with PGSE experiments.

A new STE-based PGSE NMR diffusion sequence with solvent suppression function (PGSTE-WATERGATE) was developed and its solvent suppression efficiency was illustrated on 2 mM glucose in water (10:90, $^2$H$_2$O:$^1$H$_2$O) and 2 mM lysozyme in water (10:90, $^2$H$_2$O:$^1$H$_2$O). The diffusion measuring ability of the PGSTE-WATERGATE sequence was demonstrated on the lysozyme sample.

To improve the selectivity of current WATERGATE based solvent suppression methods (i.e., excitation sculpting (e.g., excitation sculpting using the W5 sequence: $\tau$-$g_1$-$\tau$–W5–$\tau$–$g_1$–$2\tau$–$g_2$–$\tau$–W5–$\tau$–$g_2$–$\tau$) and PGSTE-WATERGATE), two Phase Modulated (PM) binomial-like sequences were developed and their solvent suppression efficiency was demonstrated on the lysozyme sample.

6.2 PGSTE-WATERGATE

6.2.1 Introduction

STE-based PGSE sequences outperform Hahn spin-echo PGSE sequences in
determining the diffusion of molecules with short transverse relaxation times (e.g., protein) and are also widely used for small molecule samples (e.g., ligands, carbohydrates). In 1996, the diffusion and relaxation editing (DIRE) sequence was developed by using a bipolar gradient pair with a soft $\pi$ pulse (i.e., a single WATERGATE unit) in a bipolar STE-based PGSE sequence [115]. However, the use of only one WATERGATE unit in an STE-based PGSE sequence may result in phase distortions caused by the application of the requisite selective pulse (the same phenomenon was found in Hahn spin-echo PGSE experiments by Hwang and Shaka [116]).

A new STE-based PGSE NMR diffusion sequence, PGSTE-WATERGATE (Figure 6–1), is presented. The new sequence provides superb solvent suppression, pure phase spectra, and also high coherence-pathway selectivity with only a 4-step phase-cycle (Figure 6–1). This sequence is complementary to the PGSE-WATERGATE sequence reported by Price et al. [96].

### 6.2.2 NMR Experiments

A 400 MHz $^1$H spectrum of a sample containing 2 mM sucrose, 0.5 mM DSS and 2 mM NaN$_3$ in water (10:90 $^2$H$_2$O:$^1$H$_2$O) was obtained at 25 ºC using the PGSTE-WATERGATE sequence. Acquisition parameters were number-of-scans = 64, $\Delta$ = 69.4 ms, and $\delta$ = 2.5 ms with the strengths of $g_1$ and $g_2$ at 0.193 T m$^{-1}$ and 0.221 T m$^{-1}$. The inter-pulse delay in the binomial pulses was set to 400 $\mu$s.

A series of 500 MHz $^1$H PGSTE-WATERGATE spectra of a sample containing 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 25 ºC. Acquisition parameters were number-of-scans = 32, $\Delta$ = 93.7 ms, and $\delta$ = 4 ms with the strengths of $g_1$ at 0.1 T m$^{-1}$ and $g_2$ varying from 0.168 T m$^{-1}$ to 0.499 T m$^{-1}$ in equal increments. The inter-pulse delay in the binomial pulses was set to 250 $\mu$s.
6.2.3 Results and Discussion

For the non-solvent resonances, the effects of the PGSTE-WATERGATE sequence can be described by the use of a transformation matrix [116]. The input and output magnetization vectors can be written as $\mathbf{m} = [m_x, m_y, m_z]'$ and $\mathbf{M} = [M_x, M_y, M_z]'$, respectively, and the transformation caused by a pulse sequence can be written as $\mathbf{M} = \mathbf{Tm}$, where $\mathbf{T}$ is a 3-by-3 matrix which represents the transformation caused by a pulse sequence and can be written as

$$
\mathbf{T} = \begin{bmatrix}
T_{xx} & T_{yx} & T_{zx} \\
T_{xy} & T_{yy} & T_{zy} \\
T_{xz} & T_{yz} & T_{zz}
\end{bmatrix}.
$$

(5.1)

The subscript for each component of $\mathbf{T}$ indicates the magnetization transformation. For example, $T_{zy}$ means a transformation from the $z$ magnetization to the $y$ magnetization. Multiplying the unit $x$ (i.e., $[1, 0, 0]'$), $y$ (i.e., $[0, 1, 0]'$), and $z$ (i.e., $[0, 0, 1]'$) magnetization vector by $\mathbf{T}$ gives

$$
\mathbf{M}_1 = \begin{bmatrix}
T_{xx} & T_{yx} & T_{zx} \\
T_{xy} & T_{yy} & T_{zy} \\
T_{xz} & T_{yz} & T_{zz}
\end{bmatrix} \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} = \begin{bmatrix} T_{xx} \\ T_{xy} \\ T_{xz} \end{bmatrix},
$$

(5.2)

and

$$
\mathbf{M}_2 = \begin{bmatrix}
T_{xx} & T_{yx} & T_{zx} \\
T_{xy} & T_{yy} & T_{zy} \\
T_{xz} & T_{yz} & T_{zz}
\end{bmatrix} \begin{bmatrix} 0 \\ 1 \\ 0 \end{bmatrix} = \begin{bmatrix} T_{yx} \\ T_{yy} \\ T_{yz} \end{bmatrix},
$$

(5.3)
Eqs. (5.2)-(5.4) indicates that the components in $T$ can be calculated by applying the sequence of interest to the unit $x$, $y$, and $z$ magnetization and calculating the output magnetization vector for each of the unit magnetization. Therefore, by the use of product operators [47], the PGSTE-WATERGATE sequence was applied to the $x$, $y$, and $z$ magnetization using the 4-step phase-cycle shown in Figure 6–1 and the transformation matrix was found as

$$
M_3 = \begin{bmatrix}
T_{xx} & T_{yx} & T_{zx} \\
T_{xy} & T_{yy} & T_{zy} \\
T_{xz} & T_{yz} & T_{zz}
\end{bmatrix}
\begin{bmatrix}
0 \\
0 \\
1
\end{bmatrix}
= \begin{bmatrix}
T_{zx} \\
T_{zy} \\
T_{zz}
\end{bmatrix}.
$$

(5.4)

This matrix shows the two important properties of the PGSTE-WATERGATE sequence: firstly, no phase factors are introduced by the application of the selective $\pi$ pulses; and secondly, the sequence gives a pure coherence-pathway (i.e., the stimulated echo) in which only half of the $z$ magnetization is transformed to $y$ magnetization.

Though it has been proved by Pelta et al. [117] that only one transient (i.e., no phase cycle) is sufficient to obtain the desired stimulated echo signal by the use of asymmetric bipolar gradient pairs (NB their phase cycle is different than that used in PGSTE-WATERGATE), a 16-step phase cycle was used to suppress residual unwanted signals and obtain accurate diffusion data in their study. The same asymmetric bipolar gradient pairs as used in the PGSTE-WATERGATE sequence
(Figure 6–1) but with \( g_1 / g_2 \) at a “magic ratio” were developed to suppress the deleterious effects of background gradients in PGSE NMR diffusion experiments [89, 110], and an 8-step phase cycle was used in ref. [89]. In the present study, because of the use of the two soft \( \pi \) pulses, a 4-step phase cycle is necessary to suppress unwanted signals and obtain proper excitation profiles (i.e., no spectral distortions).

![Figure 6–1 The PGSTE-WATERGATE sequence. The narrow bars represent \( \pi/2 \) RF pulses, \( g_1, g_2 \) and \( g_3 \) are rectangular gradient pulses with different amplitudes, and the shaped rectangles represent “W5” binomial \( \pi \) pulses [99]. The phase cycles for the first \( \pi/2 \) pulse, the first \( \pi \) pulse, the second and third \( \pi/2 \) pulses, the last \( \pi \) pulse and the receiver are \( \phi_1 = x, -x, \phi_2 = y, y, -x, \phi_3 = x, y, -x, \phi_4 = x, y, -x, \phi_5 = y, \phi_r \) (receiver phase) = \( x, -x, -x, x \). For \( \phi_2 = y \) (or \( \phi_5 = y \)), the phasing of the “W5” binomial pulse train is \( (x)_2(-x)_2 \); for \( \phi_2 = -x \), the phasing is \( (y)_3(-y)_3 \).]

The diffusion-based attenuation of the non-solvent resonances in the PGSTE-WATERGATE sequence can be written as

\[
\ln[E] = -\gamma^2 D \delta^2 \left[ \left( \Delta - \frac{4}{3} \delta - 2\delta_2 \right) (g_1 - g_2)^2 \right. \\
+ \frac{2}{3} \delta (g_1 - g_2) g_1 \\
+ \left. \left( 4\delta_2 + \frac{4}{3} \delta \right) g_1^2 \right] 
\]  
(5.6)
where, $E$ is the diffusion-based echo attenuation and $\Delta$, $\delta$, $\delta_1$, $\delta_2$, $g_1$ and $g_2$ are defined in Figure 6–1. According to Eq. (5.6), in the limit of $\Delta \gg \delta$ and $\delta_2$, the effective diffusion time for the PGSTE-WATERGATE sequence can be defined as $\Delta - 2\delta_2 - 4\delta/3$, which means the $(g_1 - g_2)g_1$ and $g_1^2$ terms in Eq. (5.6) are usually negligible in practice. However, when $\delta$ is comparable with $\Delta$, the $(g_1 - g_2)g_1$ and $g_1^2$ terms become significant and thus have to be taken into account.

The solvent resonance is unaffected by the two selective $\pi$ pulses when the transmitter frequency is set right on the solvent resonance so a normal STE-based PGSE sequence is experienced with the gradient pair in the encoding interval being inverted from that in the decoding interval. This sequence chooses the STE#2 coherence pathway as referred to by Kingsley [51], which has the same coherence level in the encoding interval and the decoding interval. The STE#2 coherence of the solvent is attenuated by molecular diffusion, and the residual coherence can be further attenuated by the phase cycle. The diffusion-based attenuation of the solvent resonance can be written as

$$\ln[E] = -\gamma^2 D \delta^2 \left[ \left( \Delta - \frac{4}{3} \delta - 2\delta_2 \right) (g_1 + g_2)^2 + \frac{2}{3} \delta (g_1 + g_2) g_1 + \left( 4\delta_2 + \frac{4}{3} \delta \right) g_1^2 \right].$$

Comparison of Eqs. (5.6) and (5.7) reveals that the solvent resonance experiences a much higher applied gradient (i.e., the $g_1 + g_2$ terms) than the resonances of interest, which experience the $g_1 - g_2$ terms. Further, most solvent molecules (e.g., $^1$H$_2$O) have far higher diffusion coefficients than the molecules of interest (e.g., protein, peptide,
carbohydrate). For a typical solvent like water, a suppression factor of $10^4$ can be easily achieved by using gradient strengths of $g_1 = 0.110$ T m$^{-1}$ (20% of the full gradient strength in our study) and $g_2 = 0.138$ T m$^{-1}$ (25% of the full gradient strength in our study) when typical parameters are $\Delta = 90.4$ ms, $\delta = 3$ ms, $\delta_1 = 0.2$ ms and $\delta_2 = 2$ ms. Thus, the NMR signals from the solvent molecules can be totally suppressed while the resonance of the molecules of interest is left almost intact.

In a typical diffusion experiment, $g_1$ and $g_2$ ($|g_1 - g_2| = 0.005$ T m$^{-1}$) should be large enough to suppress the solvent resonance, in a measurement one of $g_1$ and $g_2$ is kept constant whilst the other can be varied up to the available gradient strength to attenuate the resonances of the studied molecules. For a slowly diffusing molecule (e.g., $D = 3 \times 10^{-11}$ m$^2$ s$^{-1}$) an attenuation of 90% (i.e., with 10% of the original signal intensity remaining) can be easily achieved by varying $g_2$ from 0.138 T m$^{-1}$ to 0.65 T m$^{-1}$ with $g_1 = 0.132$ T m$^{-1}$, $\delta = 4$ ms and $\Delta = 132.4$ ms.

As mentioned above, for the PGSTE-WATERGATE sequence, the resonances of interest only see a gradient strength of $g_1 - g_2$, which is usually quite small (e.g., 0.005 T m$^{-1}$) at the beginning of a diffusion experiment, so a good signal-to-noise ratio can be obtained even with low concentration samples. In comparison, both DIRE, which uses symmetric bipolar gradient pairs and a 16-step phase cycle, and PGSE-WATERGATE require the solvent resonances, and consequently the resonances of interest, to see a gradient strength of at least 0.1 – 0.2 T m$^{-1}$ for a good solvent suppression. As this determines the smallest values of the diffusion gradients that can be used in these sequences, it results in lower signal-to-noise ratios when measuring the diffusion of low molecular weight species.

The utility of the sequence is demonstrated on samples containing sucrose (Figure 6–2) and lysozyme (Figure 6–3). As shown in Figure 6–2 and Figure 6–3, high quality water suppression was achieved without phase distortions. The lysozyme
peaks close to the water resonance are also observable, which shows the selectivity of the suppression.

Figure 6–2 A 400 MHz $^1$H spectrum of a sample containing 2 mM sucrose, 0.5 mM DSS and 2 mM NaN$_3$ in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 298 K using the PGSTE-WATERGATE sequence. Acquisition parameters were number-of-scans = 64, $\Delta = 69.4$ ms, and $\delta = 2.5$ ms with the strengths of $g_1$ and $g_2$ at 0.193 T m$^{-1}$ and 0.221 T m$^{-1}$. The inter-pulse delay in the binomial pulses was set to 400 $\mu$s.

Figure 6–3 A series of 500 MHz $^1$H PGSTE-WATERGATE spectra of a sample containing 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 298 K. Acquisition parameters were number-of-scans = 32, $\Delta = 93.7$ ms, and $\delta = 4$ ms with the strengths of $g_1$ at 0.1 T m$^{-1}$ and $g_2$ varying from 0.168 T m$^{-1}$ to 0.499 T m$^{-1}$ in equal increments. The inter-pulse delay in the binomial pulses was set to 250 $\mu$s.
Figure 6–4 500 MHz $^1$H spectra of 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 298 K acquired with (A) PGSTE-WATERGATE (number-of-scans = 32, $\Delta$ = 91.9 ms, and $\delta$ = 4 ms with the strengths of $g_1$ and $g_2$ at 0.1 T m$^{-1}$ and 0.105 T m$^{-1}$), (B) the modified PGSTE-WATERGATE (number-of-scans = 32, $\Delta$ = 91.9 ms, and $\delta$ = 4 ms with the strengths of $g_1$ and $g_2$ at 0.152 T m$^{-1}$ and 0.157 T m$^{-1}$), (C) PGSE-WATERGATE [96] (number-of-scans = 32, $\Delta$ = 14.1 ms, and $\delta$ = 3 ms with a gradient strength of 0.315 T m$^{-1}$). In each case the “$^1$H2” pulse sequence was used for the soft $\pi$ pulses. The inter-pulse delay in the binomial pulses was set to 250 $\mu$s.
To illustrate the advantage of PGSTE-WATERGATE in removing phase distortions caused by the use of selective pulses, the PGSTE-WATERGATE sequence was compared with PGSE-WATERGATE sequence and a modified PGSTE-WATERGATE sequence in which the second soft $\pi$ pulse was replaced with a hard $\pi$ pulse. To make the comparison more rigorous, “$\bar{T}T2$” $\pi$ pulses [116], well-known for causing significant spectral phase roll [116], were used for the soft pulses. As shown in Figure 6–4B and C, for the modified PGSTE-WATERGATE and PGSE-WATERGATE sequence, the use of the “$\bar{T}T2$” pulse introduced serious phase distortions since both sequences contain only one WATERGATE unit. However, as shown in Figure 6–4A, no phase distortion was observed for the PGSTE-WATERGATE sequence containing “$\bar{T}T2$” pulses due to the symmetrical nature of the PGSTE-WATERGATE sequence.

To illustrate the ability of the PGSTE-WATERGATE sequence to determine diffusion, two diffusion experiments were performed. The first diffusion experiment was performed on the residual $^1$H$^2$HO in $^2$HO at 298 K by setting the transmitter frequency 1250 Hz away from the $^1$H$^2$HO resonance frequency so that the $^1$H$^2$HO resonance was fully inverted by the binomial $\pi$ pulse. The diffusion coefficient of $^1$H$^2$HO was determined to be $1.90 \pm 0.01 \times 10^{-9}$ m$^2$ s$^{-1}$, which is in agreement with the diffusion coefficient ($1.900 \pm 0.004 \times 10^{-9}$ m$^2$ s$^{-1}$) obtained with the standard Hahn spin-echo PGSE sequence on the same sample and literature values [10]. The second diffusion experiment was performed on a sample containing 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 298 K (Figure 6–3), and a diffusion coefficient of $1.08 \pm 0.01 \times 10^{-10}$ m$^2$ s$^{-1}$ was obtained for the lysozyme. The result is in line with the diffusion coefficient ($1.04 \pm 0.02 \times 10^{-10}$ m$^2$ s$^{-1}$) obtained with the standard STE-PGSE sequence on the same sample and also literature values [12].

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6.3 Solvent Suppression Using Phase-modulated Binomial-like Sequences

6.3.1 Introduction

In combination with the WATERGATE sequence [93], binomial-like sequences provide an easy and efficient method of solvent suppression [95]. The phases of the RF pulses in binomial or binomial-like sequences are usually 0° or 180° [95, 97-99]. However, for most modern NMR spectrometers, the phases of RF pulses can be controlled very accurately (e.g., to within 0.006°), which allows the employment of arbitrarily phased RF pulses (i.e., RF pulses with phases = \( n \times (360°/65536) \), \( n = 0, 1, 2, 3, \ldots, 65536 \)). Arbitrarily phased RF pulses have been employed in jump-return pulses for solvent and fat suppression [118, 119].

![Figure 6–5](image)

Figure 6–5 The PGSTE-WATERGATE sequence with PM1 or PM2 binomial-like π pulse. The black columns represent π/2 RF pulses, and the groupings of narrow bars represent binomial-like π pulses, and \( g_1 \), \( g_2 \) and \( g_3 \) are rectangular gradient pulses with different amplitudes. The phase cycles for the first, second, third π/2 pulse, the first and second binomial-like sequences, and receiver are \( \phi_1 = x, -x; \phi_2 = x, y, -x, -y; \phi_3 = x, y, -x, -y; \phi_4 = (x)_6, (y)_6, (y)_6, (-x)_6, (-y)_6, (-y)_6; \phi_5 = (x)_6, \phi_6 = y, -y, -y, y \). The phases of the pulses of the binomial-like sequences are given by 240.969° - 320.652° - 302.794° - 122.794° - 140.652° - 60.969° + \( \phi_4 \) (or \( \phi_3 \) for PM1) and 214.524° - 304.508° - 304.530° - 124.530° - 124.508° - 34.524° + \( \phi_4 \) (or \( \phi_3 \) for PM2).
No arbitrary phase combinations have previously been employed in binomial or binomial-like sequences due to the risk of introducing significant phase distortions. However, both excitation sculpting [116] and PGSTE-WATERGATE [74] sequences can remove phase distortions caused by the use of soft pulses. Two 6-pulse PM (Phase Modulated) binomial-like sequences with arbitrary phase combinations, which outperform the widely used 3-9-19 (also referred to as W3) sequence [95, 99], have been obtained, and the new binomial-like sequences have been combined with the excitation sculpting and PGSTE-WATERGATE (Figure 6–5) sequences.

6.3.2 NMR Experiments and Simulations
CuSO₄-doped $^2$H₂O (0.01 M CuSO₄·$^5$H₂O) was placed into a magnetic susceptibility-matched (to $^2$H₂O) NMR tube.

All experiments were conducted on a Bruker 500 MHz Avance NMR spectrometer at 25 °C. The excitation sculpting profiles of the binomial-like sequences were obtained on the CuSO₄-doped $^2$H₂O with gradient strengths of 0.185 T m⁻¹ and 0.120 T m⁻¹ for the first and second bipolar gradient pairs in the excitation sculpting sequence, respectively, and an inter-pulse delay, $\tau$, of 280 μs for the binomial-like sequences. The excitation sculpting spectra of lysozyme were obtained using the excitation sculpting sequence with number-of-scans = 64, two gradient strengths of 0.203 T m⁻¹ and 0.072 T m⁻¹ in combination with different binomial-like sequences with inter-pulse delay of 282 μs. The WATERGATE spectrum of lysozyme was obtained using the WATERGATE sequence with number-of-scans = 64, a gradient strength of 0.195 T m⁻¹ in combination with the PM1 sequence with inter-pulse delay of 282 μs. PGSTE-WATERGATE experiments were performed on
lysozyme with number-of-scans = 32, \( g_1 \) varying from 0.159 T m\(^{-1} \) to 0.541 T m\(^{-1} \), \( g_2 \) = 0.131 T m\(^{-1} \), \( \delta = 4 \) ms, \( \Delta = 62.2 \) ms, \( \delta_1 = 0.05 \) ms, and \( \delta_2 = 0.2 \) ms.

Maple routine least-squares tool (LSSolve), which is based on a combined Gauss-Newton and modified Newton algorithm, was used for optimization. In all simulations, it was assumed that the power of the RF pulses was high enough to make the resonance offset effects during the application of the RF pulses negligible.

### 6.3.3 Results and Discussion

The general form of a 6-pulse phase-modulated binomial-like sequence in this study can be written as

\[
\alpha(\phi_1) - \tau - \beta(\phi_2) - \tau - \gamma(\phi_3) - \tau - \gamma(\phi_3 + 180^\circ) - \tau - \beta(\phi_2 + 180^\circ) - \tau - \alpha(\phi_1 + 180^\circ). \tag{5.8}
\]

To ensure that the on-resonance magnetization automatically goes back to the positive \( z \) axis (i.e., experiences a zero inversion), a 180° shift was added to the phase-pattern of the second half of the sequence. For the on-resonance magnetization, the rotations caused by \( \alpha(\phi_1) \), \( \beta(\phi_2) \), and \( \gamma(\phi_3) \) are cancelled by the rotations caused by \( \alpha(\phi_1 + 180^\circ) \), \( \beta(\phi_2 + 180^\circ) \), and \( \gamma(\phi_3 + 180^\circ) \), respectively.

For most of the selective \( \pi \) RF pulses in NMR pulse sequences, the magnetization immediately before the pulse is on the transverse (i.e., \( x-y \)) plane. In particular, in the WATERGATE [93] and WATERGATE-like sequences (e.g., excitation sculpting [116], PGSE-WATERGATE [96], and PGSTE-WATERGATE [74]), the magnetization right before the selective pulse has totally fanned out in the transverse plane, signifying that a selective \( \pi \) pulse has to invert the whole magnetization plane instead of just a magnetization vector. These selective \( \pi \) pulses
can be called plane rotation pulses [120]. The simplest way to simulate a plane rotation is to use a vector perpendicular to the plane to track the movement of the magnetization plane. A magnetization vector initially on the positive \( z \) axis can simply do the job. An ideal \( \pi \) pulse rotates the magnetization vector from the positive \( z \) direction to the negative \( z \) direction.

In all simulations, the performance of the binomial-like \( \pi \) pulses has been assessed by calculating the final \( z \) magnetization caused by applying the selective pulses to an initial positive \( z \) magnetization. Because \( z \) magnetization doesn’t provide any phase information, the simulation mentioned above may generate selective pulses not only providing the desired inversion profile but also causing significant phase errors. Therefore, the new selective pulses must be used in conjunction with the excitation sculpting and PGSTE-WATERGATE sequences to remove the phase errors.

In general, there are two approaches for the development of binomial-like pulses: analytical methods (e.g., Fourier transform approximation [98] and rotation operator treatment [98]) and numerical methods [99]. Since this study is focused on the development of binomial-like sequences with the optimal inversion profiles, a numerical optimization procedure based on least-squares analysis has been employed.

The desired selective inversion pulse has no effect on the on-resonance magnetization but inverts all off-resonance magnetizations inside the effective inversion width (i.e., \( z \)-magnetization \( M_z = 1 \rightarrow M_z = -1 \)). Therefore, in the simulations based on product operators, the sequence shown in sequence (5.8) has been applied to a magnetization along the positive \( z \) axis. The inter-pulse delay \( (\tau) \) of the sequence has been set to 200 \( \mu \)s to give a band width of 5000 Hz between the null points, and thus the \( z \)-component of the final magnetization has been calculated at five offset frequencies: 50 Hz, 1500 Hz, 2000 Hz, 3000 Hz, and 3500 Hz. Each of the
three pulse durations (i.e., $\alpha$, $\beta$, $\gamma$) has been manually set from 0° to 360° in increments of 30° before each optimization due to the limitation on the number of variables in the LSSolve least-squares optimization tool of Maple 11. For example, for $\alpha = 60°$, the final z-magnetization is a function of $\beta$, $\gamma$, $\phi_1$, $\phi_2$, $\phi_3$, and the offset frequency ($\Omega$) (i.e., $f(\beta, \gamma, \phi_1, \phi_2, \phi_3, \Omega)$). The sum-of-squares is given by

\[
\left( f(\beta,\gamma,\phi_1,\phi_2,\phi_3,50\text{Hz})-0 \right)^2 + \left( f(\beta,\gamma,\phi_1,\phi_2,\phi_3,1500\text{Hz})-(-1) \right)^2
\]
\[
+ \left( f(\beta,\gamma,\phi_1,\phi_2,\phi_3,2000\text{Hz})-(-1) \right)^2 + \left( f(\beta,\gamma,\phi_1,\phi_2,\phi_3,3000\text{Hz})-(-1) \right)^2.
\]

The optimal combination of $\beta$, $\gamma$, $\phi_1$, $\phi_2$, and $\phi_3$ has been found by minimizing the sum-of-squares shown above. The optimal combination of pulse durations and phases has been obtained for each of these manually set values. The sequences presented here have been selected from these combinations based on their inversion width and selectivity.

The newly obtained binomial-like sequences, PM1 and PM2, can be written as

\[
260.47°(240.969°) - \tau - 120.00°(320.652°) - \tau - 313.04°(302.794°)
\]
\[
- \tau - 313.04°(122.794°) - \tau - 120.00°(140.652°) - \tau - 260.47°(60.969°)
\]

(5.10)

and

\[
89.99°(214.524°) - \tau - 42.12°(304.508°) - \tau - 120.00°(304.530°)
\]
\[
- \tau - 120.00°(124.530°) - \tau - 42.12°(124.508°) - \tau - 89.99°(34.524°),
\]

(5.11)

respectively. The simulated inversion profiles are shown in Figure 6–6, and experimental excitation profiles for binomial-like sequences at different RF power levels are shown in Figure 6–7 and Figure 6–8. An inter-pulse delay of 280 μs was
used for the simulations and experiments and this corresponds to a bandwidth of 3571 Hz between the null points.

Figure 6–6 Simulated inversion profiles of the PM1, PM2, W5 and 3-9-19 sequences with an inter-pulse delay of 280 μs. It has been assumed that the power of RF pulses is high enough to make the resonance offset effects negligible during the application of RF pulses.

For the simulated profiles (Figure 6–6), the resulting frequency widths which provide better than 90% inversion are 2500, 2525, 2640, and 2040 Hz for the PM1, PM2, W5, and 3-9-19 sequences, respectively, corresponding to an increase in the inversion bandwidth of 460 Hz for PM1 and 485 Hz for PM2, compared with the 3-9-19 sequence. The frequency width of the suppression region which produces less than 10% of the intensity are 240, 236, 214, and 336 Hz for the PM1, PM2, W5, and 3-9-19 sequences, respectively, which are equivalent to narrowing the suppression region by 28% for PM1 and 30% for PM2, compared with the 3-9-19 sequence.
Figure 6–7 Excitation sculpting excitation profiles of the PM1, PM2, W5 and 3-9-19 sequences using CuSO$_4$-doped $^2$H$_2$O with $\gamma B_1/2\pi = 12$ kHz for binomial-like sequences and an inter-pulse delay of 280 $\mu$s.

For the experimental profiles with $\gamma B_1/2\pi = 12$ kHz for binomial-like sequences (Figure 6–7), the resulting frequency widths which provide better than 90% inversion are 2167, 2080, 1940, and 1767 Hz for the PM1, PM2, W5, and 3-9-19 sequences, respectively, corresponding to an increase in the inversion bandwidth of 400 Hz for PM1 and 313 Hz for PM2, compared with the 3-9-19 sequence. The frequency width of the suppression region which produces less than 10% of the intensity are 440, 410, 376 and 566 Hz for the PM1, PM2, W5, and 3-9-19 sequences, respectively, which are equivalent to narrowing the suppression region by 22% for PM1 and 28% for PM2, compared with the 3-9-19 sequence.
Figure 6–8 Excitation sculpting excitation profiles of the PM1, PM2, W5 and 3-9-19 sequences using CuSO₄-doped ²H₂O with $\gamma B_1/2\pi = 32$ kHz for binomial-like sequences and an inter-pulse delay of 280 $\mu$s.

For the experimental profiles with $\gamma B_1/2\pi = 32$ kHz for binomial-like sequences (Figure 6–8), the resulting frequency widths which provide better than 90% inversion are 2233, 2410, 2500 and 1890 Hz for the PM1, PM2, W5, and 3-9-19 sequences, respectively, corresponding to an increase in the inversion bandwidth of 343 Hz for PM1 and 520 Hz for PM2, compared with the 3-9-19 sequence; while the frequency width of the suppression region which produces less than 10% of the intensity is essentially unaffected by increasing the RF power.

Compared with the simulated profiles, the experimental profiles have slight intensity distortions at low RF power (Figure 6–7) and these are due to resonance offset effects during the application of RF pulses which were assumed to be
negligible in the design of the binomial-like sequences. These distortions were minimized by using high RF power for the binomial-like sequences (Figure 6–8), except for the PM1 sequence, which was found to be more susceptible to resonance offset effects due to the use of longer RF pulses. The experimental profiles give wider suppression regions due to the use of excitation-sculpting [99].

The two new sequences provide selectivity similar to that of the W5 sequence but with far shorter durations. For example, when $\gamma B_1/2\pi = 12$ kHz and $\tau = 300$ $\mu$s, the durations of the PM1, PM2, and 3-9-19 inverse pulses are 1.824, 1.617, and 1.6 ms, respectively, while the duration of the W5 inverse pulse is 2.825 ms. Therefore, the PM1 and PM2 sequences result in less signal attenuation by transverse relaxation than the W5 sequence.

Figure 6–9 The phase of the final magnetization vector at different offset frequencies for the PM1 sequence. The $^1$H WATERGATE spectrum of a sample containing 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) using a gradient strength of 0.195 T m$^{-1}$ and the PM1 sequence with an inter-pulse delay of 282 $\mu$s.

As mentioned above, only the “z” magnetization has been taken into account during all the simulations, and therefore the newly obtained sequences may introduce phase distortions. To show the phase gradient (i.e., frequency dependent phase shift),
a simulation was conducted, in which the PM1 binomial-like sequence was applied to a transverse magnetization vector shifted 45° away from the positive x axis. As shown in Figure 6–9A, the PM1 binomial-like sequence causes a significant frequency dependent phase shift. To demonstrate the phase distortions, the PM1-based WATERGATE sequence has been applied to the lysozyme sample (Figure 6–9B).

However, the excitation sculpting and PGSTE-WATERGATE sequence can remove phase distortions caused by the use of soft pulses [74, 116]. Therefore, in combination with excitation sculpting and PGSTE-WATERGATE, the utility of the new binomial-like sequences is demonstrated on the lysozyme sample. As shown in Figure 6–10, pure-phase spectra have been obtained for both of the new sequences. The faster inversion width and selectivity of the two new binomial-like sequences compared with the 3-9-19 sequence is evident by comparing the intensity of the peaks in the regions a, b, and c. In combination with PGSTE-WATERGATE, the new binomial-like sequences have been used in diffusion experiments on the lysozyme sample. As shown in Figure 6–11, both of the new binomial-like sequences show great selectivity in PGSTE-WATERGATE experiments.
A series of 500 MHz $^1$H excitation sculpting spectra of a sample containing 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 25 °C using the PM1, PM2, W5 and 3-9-19 sequences. The inter-pulse delay, $r$, in the binomial-like sequences was set to 282 μs. The signal intensity in region a (signals due to $^1$H protons), which is at the edge of each spectrum, reflects the inversion width of each binomial-like sequence. The significant signal
attenuation in this region of the 3-9-19 spectrum indicates that the 3-9-19 sequence provides a smaller inversion width than the PM1, PM2 and W5 sequences, which provide similar inversion widths. The signal intensity in regions b and c (signals due to backbone $^1$H$_a$ protons), which are right next to the water resonance, reflects the selectivity of each binomial-like sequence. In the PM1, PM2 and W5 spectra, the signal intensity in these regions are much higher than those in the 3-9-19 spectrum, indicating that the PM1, PM2, and W5 sequences are similar but more selective than the 3-9-19 sequence.

Figure 6–11 A series of 500 MHz $^1$H PGSTE-WATERGATE spectra of a sample containing 2 mM lysozyme in water (10:90 $^2$H$_2$O:$^1$H$_2$O) at 25 °C. The diffusion decays calculated from the spectra presented are also shown. The measured diffusion coefficients of lysozyme were, within experimental error, identical (i.e., $1.13 \pm 0.01 \times 10^{-10}$ m$^2$ s$^{-1}$ (PM1) and $1.15 \pm 0.01 \times 10^{-10}$ m$^2$ s$^{-1}$ (PM2)) by averaging the data obtained in three independent experiments for each sequence.
Chapter 7  

New MQ PGSE Experiments

7.1 Preamble

It has long been of scientific interest to enhance the spatial encoding efficiency of pulsed gradients by encoding MQ coherences which have higher $\gamma_{\text{eff}}$’s than that of a single quantum coherence [121-125]. In previous maximum-quantum PGSE experiments, the MQ coherence generation unit, “$90^\circ(I) - \tau - 180^\circ(I) 90^\circ(S) - \tau - 90^\circ(I)$”, was only combined with Hahn spin-echo PGSE sequences [100, 101]. However, in bio-molecular NMR (e.g., protein NMR), STE-based PGSE sequences normally considerably outperform Hahn spin-echo PGSE sequences due to their lower susceptibility to the signal loss caused by transverse relaxation.

In this study, six new MQ STE-based PGSE sequences are developed. The six new sequences are asymmetric maximum quantum STE-based PGSE (Asym-Max-PGSTE) (Figure 7–1A), symmetric maximum quantum STE-based PGSE (Sym-Max-PGSTE) (Figure 7–1B), maximum quantum spin order STE-based PGSE (Max-SpinOrder-PGSTE) (Figure 7–1C), asymmetric anti-phase STE-based PGSE (Asym-Anti-PGSTE) (Figure 7–2A), symmetric anti-phase STE-based PGSE (Sym-Anti-PGSTE) (Figure 7–2B), and anti-phase spin order STE-based PGSE (Anti-SpinOrder-PGSTE) (Figure 7–2C) sequence.
Figure 7–1 The Asym-Max-PGSTE (A), Sym-Max-PGSTE (B), and Max-SpinOrder-PGSTE sequence. Here the black bars, hollow bars, and grey rectangles represent $\pi/2$, $\pi$, and gradient pulses. In the Asym-Max-PGSTE sequence, $g_1$ and $g_2$ play a double-role of diffusion encoding and MQ coherence selection with $g_2/g_1 = (\gamma_{1\text{C}} + 3\gamma_{1\text{H}})/\gamma_{1\text{H}} = 13/4$ for $^{13}\text{C}^1\text{H}_3$, $g_2/g_1 = (\gamma_{1\text{C}} + 2\gamma_{1\text{H}})/\gamma_{1\text{H}} = 9/4$ for $^{13}\text{C}^1\text{H}_2$, and $g_2/g_1 = (\gamma_{1\text{C}} + \gamma_{1\text{H}})/\gamma_{1\text{H}} = 5/4$ for $^{13}\text{C}^1\text{H}$; in the Sym-Max-PGSTE and Max-SpinOrder-PGSTE sequences, $g_1$ is used for diffusion encoding and $g_2$ and $g_3$ are used for MQ coherence selection with $g_3/g_2 = 13/4$ for $^{13}\text{C}^1\text{H}_3$, $g_3/g_2 = 9/4$ for $^{13}\text{C}^1\text{H}_2$, and $g_3/g_2 = 5/4$ for $^{13}\text{C}^1\text{H}$. $r = 1/(2J_{^1\text{C}^1\text{H}})$. 

$\phi$
Figure 7–2 The Asym-Anti-PGSTE (A), Sym-Anti-PGSTE (B), and Anti-SpinOrder-PGSTE (C) sequence. Here the black bars, hollow bars, and grey rectangles represent $\pi/2$, $\pi$, and gradient pulses. In the Asym-Anti-PGSTE sequence, $g_1$ and $g_2$ play a double-role of diffusion encoding and MQ coherence selection with $g_2/g_1 = (3\gamma_{1H}/\gamma_{1H}) = 3$ for $^{13}\text{C}^{1}\text{H}_3$, $g_2/g_1 = (2\gamma_{1H} + \gamma_{13C})/\gamma_{1H} = 9/4$ for $^{13}\text{C}^{1}\text{H}_2$, and $g_2/g_1 = \gamma_{1H}/\gamma_{1H} = 1$ for $^{13}\text{C}^{1}\text{H}$; in the Sym-Anti-PGSTE and Anti-SpinOrder-PGSTE sequence, $g_1$ is used for diffusion encoding and $g_2$ and $g_3$ are used for MQ coherence selection with $g_3/g_2 = 3$ for $^{13}\text{C}^{1}\text{H}_3$, $g_3/g_2 = 9/4$ for $^{13}\text{C}^{1}\text{H}_2$, $g_3/g_2 = 1$ for $^{13}\text{C}^{1}\text{H}$, and $\tau = \tau' = 1/(2J_{13C^{1}H})$. 
Here ‘asymmetric’ means the MQ coherences at two different coherence levels are encoded during the two encoding intervals, respectively, ‘symmetric’ means the MQ coherences at the same coherence level are encoded during the two encoding intervals, ‘maximum quantum’ means MQ coherences with the maximum coherence level (e.g., [102]) is involved in diffusion encoding, ‘anti-phase’ means the anti-phase MQ coherences are encoded, and ‘spin order’ means spin order is used for phase storage. The MQ coherence selectivity and diffusion measuring ability of the new sequences were illustrated with L-[3,13C]-alanine.

7.2 Sample Preparation and 1D and Diffusion Experiments

A solution of L-[3,13C]-alanine (9.6 mM) was prepared by dissolving 4.3 mg L-[3,13C]-alanine in 5 mL 2H2O.

1H spectra of L-[13C]-alanine were obtained using new sequences with the MQ coherence selection gradient amplitude ratios (i.e., g1/g2 for the Asym-Max-PGSTE sequence shown in Figure 7–1A and the Asym-Anti-PGSTE sequence shown in Figure 7–2A and g2/g3 for the other sequences shown in Figure 7–1 and Figure 7–2 set to 0.1, 0.2, 4/13, 4/9, 0.5, 0.6, 0.7, 0.8, 0.9, 1 (for the maximum-quantum sequences) or 0.1, 0.2, 1/3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 (for the anti-phase sequences).

MQ diffusion experiments were performed by varying g2 (for the Asym-Max-PGSTE and Asym-Anti-PGSTE sequence) from 0.02 to 0.51 T m⁻¹ or g1 (for other sequences) from 0.01 to 0.21 T m⁻¹ with the MQ coherence selection gradient amplitude ratio set to 4/13 (for the maximum-quantum coherence encoding sequences) or 1/3 (for the anti-phase coherence encoding sequences) to select the QQ or TQ coherence of 13C1H3, respectively.

The GARP sequence was used for decoupling 13C and 1H during acquisition.
7.3 Results and Discussion

\( \gamma_{\text{eff}} \) enhancement versus signal loss

Before the MQ STE-based PGSE sequences can be developed, two important questions have to be answered: 1. Is it compulsory to always generate the coherences with maximum coherence level to maximize not only the encoding efficiency of applied gradients but also the signal loss in coherence selection? 2. Should longitudinal magnetization (e.g., \(^1\text{H}, ^{15}\text{N}, \) or \(^{13}\text{C}\)) or spin order (e.g., \(^1\text{H}^1\text{H}^1\text{H}^{13}\text{C}\)) be chosen for storing phase information during the storage period?

In previous \( \gamma_{\text{eff}} \) enhancement PGSE experiments, researchers were trying to generate \( n \)-spin coherences (e.g., \( 8I_yI_xS_y \) for \(^{13}\text{C}^1\text{H}_3\)) and thus maximum-quantum coherence levels to maximize \( \gamma_{\text{eff}} \). However, the application of pulsed gradients on an \( n \)-spin coherence (e.g., \( 8I_yI_xI_yS_y \)) can not only enhance \( \gamma_{\text{eff}} \) but also cause significant signal loss. For example, the application of pulsed gradients on \( 8I_yI_xI_yS_y \) generates 8 different product operator terms (i.e., 8 different coherences) [100]:

\[
8 \left( I_x \cos \theta^I_e + I_y \sin \theta^I_e \right) \left( I_y \cos \theta^I_e - I_x \sin \theta^I_e \right) \\
\times \left( I_y \cos \theta^I_e - I_y \sin \theta^I_e \right) \left( S_y \cos \theta^S_e - S_x \sin \theta^S_e \right) \\
= 8I_yI_yS_y \left( \cos^2 \theta^I_e - 2 \sin^2 \theta^I_e \right) \cos \theta^I_e \cos \theta^S_e \\
- 8I_yI_yI_xS_y \left( \cos^2 \theta^I_e - 2 \sin^2 \theta^I_e \right) \cos \theta^I_e \cos \theta^S_e \\
- 8I_yI_yI_xS_y \left( 2 \cos^2 \theta^I_e - \sin^2 \theta^I_e \right) \sin \theta^I_e \cos \theta^S_e \\
+ 8I_yI_yI_xS_y \left( 2 \cos^2 \theta^I_e - \sin^2 \theta^I_e \right) \sin \theta^I_e \sin \theta^S_e \\
+ 8I_yI_yI_xS_y \cos \theta^I_e \sin^2 \theta^I_e \cos \theta^S_e \\
- 8I_yI_yI_xS_y \cos \theta^I_e \sin^2 \theta^I_e \sin \theta^S_e \\
+ 8I_yI_yI_xS_y \cos \theta^I_e \sin^2 \theta^I_e \cos \theta^S_e \\
- 8I_yI_yI_xS_y \cos \theta^I_e \sin^2 \theta^I_e \sin \theta^S_e ,
\]

(6.1)

where \( \theta^I_e \) and \( \theta^S_e \) are the phase shifts caused by the application of the encoding gradient pulses for spins \( I \) (e.g., \(^1\text{H}\)) and \( S \) (e.g., \(^{13}\text{C}\)), respectively. Only one of these
8 terms (e.g., the $8I_xI_yS_z$ term) can be selected in one transient. While, the application of pulsed gradients on $8I_xI_yS_z$ (i.e., an anti-phase coherence) only generates 4 different coherences:

$$8\left(I_x\cos\theta' + I_y\sin\theta'\right)\left(I_y\cos\theta' - I_x\sin\theta'\right)$$

$$\times(\left(I_y I_x S_z\right)\left(2\sin^2\theta' \cos\theta'\right)$$

$$+ 8I_x I_y S_z \left(\sin^3\theta' - 2\sin^2\theta' \cos^2\theta'\right)$$

$$+ 8I_x I_y S_z \sin^2\theta' \cos\theta'$$

$$+ 8I_y I_x S_z \sin\theta' \cos^2\theta',$$

and one of these 4 coherences (e.g., $8I_xI_yS_z$) can be selected in one transient, indicating that less signal loss will be encountered if there are less $I_v$'s or $S_v$'s in the coherence. Therefore, it is important to find the best trade-off between $\gamma_{eff}$-enhancement and signal loss in practice, that is only the $I_v$ or $S_v$ term which can generate a significant increase in $\gamma_{eff}$ should be generated in the coherence. For instance, for the QQ coherence $8^{1}H_{x}^{1}H_{y}^{1}H_{z}^{13}C_y\gamma_{eff} = \gamma_{1H} + \gamma_{1H} + \gamma_{1H} + \gamma_{13C} = 13\gamma_{1H}/4$ while for the TQ coherence $8^{1}H_{x}^{1}H_{y}^{1}H_{z}^{13}C_z\gamma_{eff} = 3\gamma_{1H}$, and it is easy show that the involvement of $^{13}C_y$ only provides a $(13\gamma_{1H}/4 - 3\gamma_{1H})/(3\gamma_{1H}) \approx 8\%$ increase in the $\gamma_{eff}$ but causes significantly higher signal loss as indicated by comparing Eq. (6.1) and (6.2). In the present study, $n$-spin coherences and $n$-spin anti-phase coherences were both used for diffusion encoding.

Different types of longitudinal magnetization or spin order have different longitudinal relaxation times. To reduce the signal attenuation caused by longitudinal relaxation and thus maximize the available diffusion time, $^{15}N_z$ was used to store phase information in the X-STE sequence due to its far longer longitudinal relaxation time (e.g., 1 s) than the longitudinal relaxation time of longitudinal proton
magnetization (e.g., 100 ms) [126]. In this study, longitudinal magnetization $^1\text{H}_z$ and spin orders (e.g., $^8\text{H}_z^1\text{H}_z^1\text{H}_z^1\text{C}_z^{13}$) were used for phase information storage.

**Newly developed MQ STE-based PGSE sequences**

The evolutions of the product operators for the three maximum quantum STE-based PGSE sequences are given here; in the equations, $S$ stands for $^{13}\text{C}$, $X$ stands for $^{12}\text{C}$, and $I$ stands for $^1\text{H}$. As shown by Eqs. (6.1) and (6.2), the application of pulsed gradients on a MQ coherence generates more than one coherences; however, only the desired coherences after the application of pulsed gradients are shown for all the new MQ sequences. For the Asym-Max-PGSTE (Figure 7–1A), the evolution of product operators after the “$90^\circ_e(I)−1/(2J)−180^\circ_e(I)90^\circ_e(S)−1/(2J)−90^\circ_e(I)$” unit may be written as

For the Sym-Max-PGSTE sequence (Figure 7–1B), the evolution of product operators after the “$90^\circ_e(I)−1/(2J)−180^\circ_e(I)90^\circ_e(S)−1/(2J)−90^\circ_e(I)$” unit may be written as
For the Max-SpinOrder-PGSTE sequence, the evolution of product operators after the “$90_y^0(I) - 1/(2J) - 180_y^0(I) 90_y^0(S) - 1/(2J) - 90_y^0(I)$” unit may be written as

\[
\begin{align*}
SI_1 & : -2I_yS_y & e^{-180_y^0(I) 90_y^0(S)} & \rightarrow -2I_yS_y & 90_y^0(I) & \rightarrow -2I_yS_y \\
SI_2 & : -4I_yI_yS_y & +4I_yI_yS_y & +4I_yI_yS_y \\
SI_3 & : +8I_yI_yI_yS_y & +8I_yI_yI_yS_y & +8I_yI_yI_yS_y \\
XI_n & : +I_z & -I_z & +I_z \\
\end{align*}
\]

(6.4)

For the Asym-Anti-PGSTE (Figure 7–2A) sequence, the evolution of product operators after the “$90_y^0(I) - 1/(2J) - 180_y^0(I) 90_y^0(S) - 1/(2J) - 90_y^0(I)$” unit may be written as

\[
\begin{align*}
SI_1 & : -2I_yS_y & e^{-180_y^0(I) 90_y^0(S)} & \rightarrow +2I_yS_y & 90_y^0(I) & \rightarrow +2I_yS_y \\
SI_2 & : -4I_yI_yS_y & +4I_yI_yS_y & +4I_yI_yS_y \\
SI_3 & : +8I_yI_yI_yS_y & -8I_yI_yI_yS_y & -8I_yI_yI_yS_y \\
XI_n & : +I_z & -I_z & +I_z \\
\end{align*}
\]

(6.5)

For the Asym-Anti-PGSTE (Figure 7–2A) sequence, the evolution of product operators after the “$90_y^0(I) - 1/(2J) - 180_y^0(I) 90_y^0(S) - 1/(2J)$” unit may be written as
For the Sym-Anti-PGSTE sequence (Figure 7–2B), the evolution of product operators after the “90° \(I\)–1/(2\(J\))–180° \(S\)–1/(2\(J\))” unit may be written as

\[
\begin{align*}
SI_1 & : -2I_x S_y & 90°(I) & -2I_x S_y \\
SI_2 & : -4I_x I_y S_y & +4I_x I_y S_y \\
SI_3 & : +8I_x I_y S_y & +8I_x I_y S_y \\
XI_x & : +I_y & +I_y \\
\end{align*}
\]

\[
\begin{align*}
\rightarrow & +2I_y S_x \\
\rightarrow & +4I_y S_x \\
\rightarrow & +8I_y S_x \\
+ & +I_y \\
- & +I_y \\
- & +I_y \\
\end{align*}
\]

(6.6)

For the Anti-SpinOrder-PGSTE sequence (Figure 7–2C), the evolution of product operators after the “90° \(I\)–1/(2\(J\))–180° \(I\)90° \(S\)–1/(2\(J\))” unit may be written as

\[
\begin{align*}
SI_1 & : -2I_x S_y & 90°(I) & -2I_x S_y \\
SI_2 & : -4I_x I_y S_y & +4I_x I_y S_y \\
SI_3 & : +8I_x I_y S_y & +8I_x I_y S_y \\
XI_x & : +I_y & +I_y \\
\end{align*}
\]

\[
\begin{align*}
\rightarrow & +2I_y S_x \\
\rightarrow & +4I_y S_x \\
\rightarrow & +8I_y S_x \\
+ & +I_y \\
- & +I_y \\
- & +I_y \\
\end{align*}
\]

(6.7)
The main features of the six new sequences are shown in Table 7-1. As this study focused on the MQ coherences of $^{13}$C$^1$H$_3$ which provides the highest $\gamma_{\text{eff}}$ enhancement, only the features related to the selection of these MQ coherences are listed in Table 7-1. As shown in Table 7-1, for the Aysm-Max-PGSTE, Sym-Max-PGSTE, and Max-SpinOrder-PGSTE sequences, the DQ and QQ coherences [102] are selected when $g_1/g_2$ or $g_2/g_3$ is set to 4/5 and 4/13, respectively; for the Aysm-Anti-PGSTE, Sym-Anti-PGSTE, and Anti-SpinOrder-PGSTE sequences, the single quantum and TQ coherences are selected when $g_1/g_2$ or $g_2/g_3$ is set to 1 and 1/3, respectively.
Table 7-1 Main features of the Asym-Max-PGSTE, Sym-Max-PGSTE, Max-SpinOrder-PGSTE, Asym-Anti-PGSTE, Sym-Anti-PGSTE, and Anti-SpinOrder-PGSTE.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Encoded Terms</th>
<th>MQ coherence selected</th>
<th>g1/g2 or g2/g3</th>
<th>Phase storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asym-Max-PGSTE</td>
<td>8(^1)H(_x), (1)H(_y), 13(^{13})C(_y)</td>
<td>DQ</td>
<td>4/5</td>
<td>(^1)H(_z)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QQ</td>
<td>4/13</td>
<td></td>
</tr>
<tr>
<td>Sym-Max-PGSTE</td>
<td>8(^1)H(_x), (1)H(_y), 13(^{13})C(_y)</td>
<td>DQ</td>
<td>4/5</td>
<td>(^1)H(_z)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QQ</td>
<td>4/13</td>
<td></td>
</tr>
<tr>
<td>Max-SpinOrder PGSTE</td>
<td>8(^1)H(_x), (1)H(_y), 13(^{13})C(_y)</td>
<td>DQ</td>
<td>4/5</td>
<td>8(^1)H(_x), (^1)H(_z), 13(^{13})C(_z)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QQ</td>
<td>4/13</td>
<td></td>
</tr>
<tr>
<td>Asym-Anti-PGSTE</td>
<td>8(^1)H(_x), (1)H(_y), 13(^{13})C(_z)</td>
<td>Single quantum</td>
<td>1</td>
<td>(^1)H(_z)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TQ</td>
<td>1/3</td>
<td></td>
</tr>
<tr>
<td>Sym-Anti-PGSTE</td>
<td>8(^1)H(_x), (1)H(_y), 13(^{13})C(_z)</td>
<td>Single quantum</td>
<td>1</td>
<td>(^1)H(_z)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TQ</td>
<td>1/3</td>
<td></td>
</tr>
<tr>
<td>Anti-SpinOrder PGSTE</td>
<td>8(^1)H(_x), (1)H(_y), 13(^{13})C(_z)</td>
<td>Single quantum</td>
<td>1</td>
<td>8(^1)H(_x), (^1)H(_z), 13(^{13})C(_z)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TQ</td>
<td>1/3</td>
<td></td>
</tr>
</tbody>
</table>

MQ coherence selectivity

The MQ coherence selectivity of each new MQ STE-based PGSE sequence was illustrated with L-[3-\(^{13}\)C]-alanine. As shown in Figure 7–3, the QQ coherence of \(^{13}\)C\(^1\)H\(_3\), which provides the highest \(\gamma_{\text{eff}}\)-enhancement, was successfully selected by using the Asym-Max-PGSTE, Sym-Max-PGSTE, and Max-SpinOrder-PGSTE sequences with the amplitudes of the MQ coherence selecting gradient pairs at a ratio of 4:13. It is also shown that the Asym-Max-PGSTE sequence provided a significantly higher S/N ratio than the Sym-Max-PGSTE and Max-SpinOrder-PGSTE sequences because the Asym-Max-PGSTE sequence, in which the MQ coherences
were encoded only once, suffered far less signal loss due to MQ coherences encoding (e.g., Eq. (6.1)) than the Sym-Max-PGSTE and Max-SpinOrder-PGSTE sequences, in which the MQ coherences were encoded twice. Finally, the S/N ratio of the Max-SpinOrder-PGSTE spectra was much lower than that of the Sym-Max-PGSTE spectra possibly due to the much shorter longitudinal relaxation time of $^{1}H_{z}^{1}H_{z}^{1}H_{z}{^{13}}C_{z}$ than that of $^{1}H_{z}$; side bands were observed around some of the DQ and QQ peaks due to incomplete decoupling during the acquisition.

As shown in Figure 7–4, the TQ coherence was successfully selected by using the Asym-Anti-PGSTE and Anti-SpinOrder-PGSTE sequences with the amplitudes of the MQ coherence selecting gradient pairs at a ratio of 1:3 and the Asym-Anti-PGSTE sequence provided a higher S/N ratio. Interestingly, the Anti-SpinOrder-PGSTE sequence provided a higher S/N ratio than the Max-SpinOrder-PGSTE sequence as proposed at the beginning of this Section while the Asym-Anti-PGSTE provided no S/N enhancement compared with the Asym-Max-PGSTE sequence due to the effects of $J$-evolution.
Figure 7–3 \(^1\)H spectra of L-[\(^{13}\)C]-alanine in \(^2\)H\(_2\)O using (A) the Asym-Max-PGSTE, (B) Sym-Max-PGSTE, and (C) Max-SpinOrder-PGSTE sequences. For the Asym-Max-PGSTE sequence, \(g_1/g_2 = 0.1, 0.2, 4/13, 4/9, 0.5, 0.6, 0.7, 0.8, 0.9, \) and 1; for the Sym-Max-PGSTE and Max-SpinOrder-PGSTE sequences, \(g_2/g_3 = 0.1, 0.2, 4/13, 4/9, 0.5, 0.6, 0.7, 0.8, 0.9, \) and 1. The \(^{13}\)C\(^1\)H peaks are around 1.4 ppm and the \(^{12}\)C\(^1\)H peaks are around 3.7 ppm.
Figure 7-4 \(^1\)H spectra of L-[3\(^{13}\)C]-alanine in \(^2\)H\(_2\)O using (A) the Asym-Anti-PGSTE, (B) Sym-Anti-PGSTE, and (C) Anti-SpinOrder-PGSTE sequences. For the Asym-Anti-PGSTE sequence, \(g_1/g_2 = 0.1, 0.2, 1/3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, \) and 1; for the Sym-Anti-PGSTE and Anti-SpinOrder-PGSTE sequences, \(g_2/g_3 = 0.1, 0.2, 1/3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, \) and 1. The \(^{13}\)C\(^1\)H\(_3\) peaks are around 1.4 ppm and the \(^{12}\)C\(^1\)H peaks are around 3.7 ppm.
Diffusion measurements

Diffusion measurements were performed on the L-\([^{13}\text{C}]\)-alanine sample by the use of all the newly developed MQ STE-based PGSE sequences except the Sym-Anti-PGSTE sequence which had a poor TQ coherence selectivity (Figure 7–4B). As shown in Figure 7–5, both the Asym-Max-PGSTE and Sym-Max-PGSTE sequences provided good diffusion decays while the Max-SpinOrder-PGSTE sequence provided a poor diffusion decay due to a poor S/N ratio. It can also be seen in Figure 7–5 that for each sequence the signal intensity of the first experiment was lower than that of the second experiment indicating that the starting intensities of the diffusion encoding gradients (i.e., \(g_1\) and \(g_2\) for the Asym-Max-PGSTE and Asym-Anti-PGSTE sequence and \(g_1\) for the Sym-Max-PGSTE, Max-SpinOrder-PGSTE, Sym-Anti-PGSTE, and Anti-SpinOrder-PGSTE sequence) should be high enough to select the desired MQ coherences.

The diffusion measurements using the Asym-Anti-PGSTE and Anti-SpinOrder-PGSTE sequences are shown in Figure 7–6. The Asym-Anti-PGSTE sequence provided a good diffusion decay while the Anti-SpinOrder-PGSTE sequence provided a poor diffusion decay due to a poor S/N ratio.
Figure 7–5 $^1$H spectra of L-[3-$^{13}$C]-alanine in $^2$H$_2$O with different diffusion encoding gradient values using (A) the Asym-Max-PGSTE sequence with $g_1/g_2 = 4/13$, (B) the Sym-Max-PGSTE sequence with $g_2/g_3 = 4/13$, and (C) the Max-SpinOrder-PGSTE sequence with $g_2/g_3 = 4/13$. 
Figure 7–6 $^1$H spectra of L-[\textit{3-}$^{13}$C]-alanine in $^2$H$_2$O with different diffusion encoding gradient values using (A) the Asym-Anti-PGSTE sequence with $g_1/g_2 = 1/3$ and (B) the Anti-SpinOrder-PGSTE sequence with $g_2/g_3 = 1/3$. 
8.1 Suppression of the Effects of Nonconstant Background Gradients

Due to its higher efficiency in suppressing both the cross-terms and $g_0^2$ terms, the MAG-PGSTE sequence outperformed the MAGSTE and Cotts 13-interval sequences in diffusion measurements at different diffusion times in the presence of large and highly inhomogeneous background gradients in the $< 106 \, \mu m$ glass bead sample; while, for the $212 - 300 \, \mu m$ glass bead sample, the MAGSTE sequence provided better diffusion measurements than the other two sequences. Therefore, the MAG-PGSTE sequence is more suitable for the suppression of the deleterious effects of large and highly inhomogeneous background gradients.

It was also observed that at long diffusion times (e.g., $\Delta > 100$ ms) the MAG-PGSTE diffusion measurements were significantly higher than the MAGSTE and Cotts 13-interval diffusion measurements, signifying that the MAG-PGSTE sequence may perform better in diffusion diffraction experiments (with long diffusion times like a few seconds), which can be significantly affected by background gradients [127, 128].

The MAG-PGSTE sequence also provided accurate sphere size characterizations using long pulsed gradients on the high resolution probe. For the MAG-PGSTE and MAGSTE sequences, the use of long and less intensive pulsed
gradients (i.e., \( \delta = 3 \) ms) provided better sphere size characterizations than the use of short and intensive pulsed gradients (i.e., \( \delta = 0.5 \) ms), indicating that long pulsed gradients should be preferred to short pulsed gradients in the presence of inhomogeneous background gradients. The possible reason for this is that the average intensity of the fluctuating background gradient during the application of each short and intensive pulsed gradient may be significantly different while the average intensity of the background gradient during the application of each long and less intensive pulsed gradient may be less different due to the average over a longer time interval; therefore, the assumption of a constant \( g_0 \) during each encoding period is more applicable for the use of long pulsed gradients. It was also observed that using long encoding periods provided poor attenuation measurements. Consequently, long pulsed gradients and short encoding periods are preferred for measuring diffusion in the presence of non-constant background gradients that is the encoding periods should be “filled” up with pulsed gradients as much as possible.

### 8.2 Solvent Suppression in PGSE Experiments

The PGSTE-WATERGATE sequence provides superb solvent suppression and pure phase spectra in PGSE experiments. The sequence is simple to set up and particularly suited to measuring diffusion in aqueous solution such as is commonly required in pharmaceutical and combinatorial applications. Importantly, the high degree of phase-distortion suppression allows more sophisticated selective \( \pi \) pulses to be used to enhance the selectivity of solvent suppression.

In combination with excitation sculpting, both of the phase modulated binomial-like sequences (i.e., PM1 and PM2) outperform the 3-9-19 sequence in selectivity and inversion width. With significantly shorter durations, the PM1 and
PM2 sequences provide similar selectivity and inversion width to the W5 sequence. Combining the PM1 and PM2 sequences with the PGSTE-WATERGATE sequence, highly selective solvent suppression can be achieved in PGSE diffusion experiments.

The evolution of the water resonance during the PGSTE-WATERGATE sequence and the PM1 and PM2 sequences will be studied in detail and the effects of long range dipole-dipole interactions will be included. This study will help us improve the current sequences for the water suppression in low concentration samples.

### 8.3 MQ PGSE Experiments

The QQ coherences from $^{13}$C$_1$H$_3$ can be successfully selected by the use of the Asym-Max-PGSTE, Sym-Max-PGSTE, and Max-SpinOrder-PGSTE sequences; the TQ coherences from $^{13}$C$_1$H$_3$ can be successfully selected by the use of the Asym-Anti-PGSTE and Anti-SpinOrder-PGSTE sequences. Therefore, the Asym-Max-PGSTE and Asym-Anti-PGSTE sequences can be used for diffusion and MQ coherence editing; by the use of the Sym-Max-PGSTE, Max-SpinOrder-PGSTE, and Anti-SpinOrder-PGSTE sequences, significant $\gamma_{\text{eff}}$-enhancement can be achieved in PGSE diffusion experiments.

Anti-SpinOrder-PGSTE sequence provides a higher S/N ratio than the Max-SpinOrder-PGSTE sequence while the Asym-Anti-PGSTE sequence doesn’t provide any S/N enhancement against the Asym-Max-PGSTE sequence due to the effects of $J$-evolution.

### 8.4 Directions for Future Research

The incomplete suppression of background gradients by both the MAG-PGSTE and MAGSTE sequences indicates that in extremely magnetically inhomogeneous
systems like water saturated glass bead packs the background gradients fluctuate during the encoding period; therefore, the STE-based PGSE methods, which can suppress the deleterious effects of the fast fluctuating background gradients, will be of great interest. The development of the new suppression methods should following two guide lines: 1. Encoding periods must be short and filled with pulsed gradients; 2. pulsed gradients with arbitrary shapes may be used.

Current solvent suppression techniques can easily kill the solvent signal for the samples with relatively high concentrations (e.g., 5 mM). However, many physiological systems contain bio-molecules at very low concentrations (e.g., 0.05 mM) so new solvent suppression techniques need to be developed for the solvent suppression for low concentration samples. The combination of different suppression methods (e.g., relaxation-based suppression + diffusion-based suppression) may be a possible way of suppressing solvent signal for low concentration samples. Also, combining the PGSTE-WATERGATE method with multiple selective inversion pulses may provide new ways of multiple solvent suppression.

Although the diffusion measurement on slow diffusing species (e.g., protein aggregate) can be significantly improved by the use of MQ encoding, it can be further improved by combining MQ encoding with the generation of long-life phase storage species such as singlet state.
APPENDIX A Pulse Programs

MAG-PGSTE

; MAG-PGSTE_1D (the gradients in the decoding interval have
been inverted, delta1=delta2)
; G. Zheng and W.S. Price 06 July 2008
; for Avance 500

; 1D stimulated spin echo with asymmetric bipolar gradients
for diffusion measurements in
; the presence of background gradients
;
#include <Avance.incl>
#include <Grad.incl>
"d22 = 50u";
"p2 = p1 * 2"
"p20 = p16/2"
"d18 = ( 2*d17 + p20 + p2 + d22*2) * 0.5 - p1 * 1.5"

1 ze
2 d1
p1 ph1
   d18
p2 ph4
   d22 UNBLKGRAMP
   d17
   p20:gp1
   d17
   d22 BLKGRAMP
p2 ph4
   d22 UNBLKGRAMP
   d17
   p20:gp1*η
   d17
   d22 BLKGRAMP
p2 ph4
   d18
p1 ph2
   d22 UNBLKGRAMP
   p17:gp3
   d17
   d22 BLKGRAMP
   d25
p1 ph3
d18
p2 ph5
d22 UNBLKGRAMP
d17
p20:gp1* η
d17
d22 BLKGRAMP
p2 ph5
d22 UNBLKGRAMP
d17
p20:gp1
d17
d22 BLKGRAMP
p2 ph5
d18
go=2 ph31
wr #0
exit

ph1  = 0
ph2  = 0 2 0 2 1 3 1 3
ph3  = 0 0 2 2 1 1 3 3
ph4  = 1
ph5  = 1 1 3 3 2 2 0 0
ph31 = 0 2 2 0 2 0 0 2

;RF PULSES
;p11 : f1 channel - power level for pulse (default) usually 2.65 dB
;p1 : f1 channel - 90 degree high power pulse ~8-9 us

;DELAYS
;d1 : relaxation delay 1-5 * T1
;d15 : tau2
;d17 : gradient recovery delay (~ 100 us should be enough for high res probe)

;GRADIENTS
;p16: diffusion gradient pulse = little delta [1-5 ms]
; gradient shape is controlled by gpnam1
; gradient amplitude is controlled by gpz1,gpz2
;p17: purge gradient pulse in tau2 = typically [1-2 ms]
; gradient shape is controlled by gpnam3
; gradient amplitude is controlled by gpz3

;SCANS AND PHASE CYCLES
;NS: preferably 8 * n
;use the au program wp_diffamp to increment the gradients
PGSTE-WATERGATE

; PGSTE-WATERGATE
; Gang Zheng, Timothy Stait-Gardner, P.G. Anil Kumar, Allan M. Torres, William S. Price 12 Mar 2008 (Based on Price's initial idea)

; for Avance 500
; 1D STE-based PGSE sequence with water suppression
; locked version

#include <Avance.incl>
#include <Grad.incl>

"p2 = p1 * 2"
"p20 = p16/2"

1 ze
30u
2 d1 pl1:f1
p1 ph1
50u UNBLKGRAD
p20:gp1 ; 1st grad pulse of 1st bpg
50u UNBLKGRAD
p17:gp3 
50u BLKGRAD
50u UNBLKGRAD
p17:gp3 
50u BLKGRAD

; change power level back
; 2nd grad pulse of 1st bpg, end of

WaterGate

50u
p28*0.087 ph4
d19*2
p28*0.206 ph4
d19*2
p28*0.413 ph4
d19*2
p28*0.778 ph4
d19*2
p28*1.491 ph4
d19*2
p28*1.491 ph5
d19*2
p28*0.778 ph5
d19*2
p28*0.413 ph5
d19*2
p28*0.206 ph5
d19*2
p28*0.087 ph5
50u pl1:f1
50u p11:f1
50u UNBLKGRAD
p20:gp2 
50u BLKGRAD

; change power to level p118 for
; 1st grad pulse of 1st bpg

WaterGate

d17

50u BLKGRAD

p1 ph2

50u UNBLKGRAD

50u BLKGRAD
d25
p1 ph3
50u UNBLKGRAD
p20:gp2*-1
d17 p118:f1 ; change power to level p118 for WaterGate
50u
p28*0.087 ph6
d19*2
p28*0.206 ph6
d19*2
p28*0.413 ph6
d19*2
p28*0.778 ph6
d19*2
p28*1.491 ph6
d19*2
p28*1.491 ph7
d19*2
p28*0.778 ph7
d19*2
p28*0.413 ph7
d19*2
p28*0.206 ph7
d19*2
p28*0.087 ph7
50u
p20:gp1*-1
d17
50u BLKGRAD
go=2 ph31
wr #0
exit

ph1 = 0 2
ph2 = 0 1 2 3
ph3 = 0 1 2 3
ph4 = 0 0 1 1 2 2 3 3
ph5 = 2 2 3 3 0 0 1 1
ph6 = 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
ph7 = 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3
0 0 0 0 0 0 0 0 1 1 1 1 1 1 1
ph31 = 0 2 2 0 0 2 2 0 2 0 0 2 2 0 0 2

; RF PULSES
; p11 : f1 channel - power level for pulse (default) usually 2.65 dB
; p118 : f1 channel - power level for WaterGate pulse. This value can be calibrated using ; calcpowlev
;
; p1 : f1 channel - 90 degree high power pulse ~8 us
; p28 : f1 channel - 90 degree low power pulse for watergate (~ 21us)
; DELAYS
; d1 : relaxation delay 1-5 * T1
; d15 : Captital delta
; d17 : gradient recovery delay (~ 100 us should be enough for high resolution probe)

; GRADIENTS
; p16: diffusion gradient pulse [1-8 ms]
; gradient shape is controlled by gpnam1 and gpnam2
; gradient amplitude is controlled by gpz1 and gpz2 (gpz1>gpz2)
;
; p17: purge gradient pulse in tau 2 = typically [1-2 ms]
; gradient shape is controlled by gpnam3
; gradient amplitude is controlled by gpz3

; SCANS AND PHASE CYCLES
; NS: preferably 8 * n
; use the au program wp_diffamp to increment the gradients
Excitation sculpting using PM1

; Excitation sculpting using PM1
; 1D sequence

#include <Avance.incl>
#include <Grad.incl>

1 ze
30u
2 d1 p11:f1
   p1 ph1
d16 UNBLKGRAD
   p16:gp1
d16 p118:f1
   p28*2.894120913 ph3
d19*2
   p28*1.333333333 ph4
d19*2
   p28*3.478208604 ph5
d19*2
   p28*3.478208604 ph6
d19*2
   p28*1.333333333 ph7
d19*2
   p28*2.894120913 ph8
d16
   p16:gp1
d16
d16
   p16:gp2
d16
   p28*2.894120913 ph9
d19*2
   p28*1.333333333 ph10
d19*2
   p28*3.478208604 ph11
d19*2
   p28*3.478208604 ph12
d19*2
   p28*1.333333333 ph13
d19*2
   p28*2.894120913 ph14
d16
   p16:gp2
d16
4u BLKGRAD
   go=2 ph31
   wr #0
exit

ph1=0 2
ph3=(65536) 43867 43867 60251 60251 11099 11099 27483 27483
ph4=(65536) 58373 58373  9221  9221 25605 25605 41989 41989
ph5=(65536) 55122 55122  5970  5970 22354 22354 38738 38738
ph6=(65536) 22354 22354 38738 38738 55122 55122  5970  5970
ph7=(65536) 25605 25605 41989 41989 58373 58373  9221  9221
ph8=(65536) 11099 11099 27483 27483 43867 43867 60251 60251
ph9=(65536) 43867
ph10=(65536) 58373
ph11=(65536) 55122
ph12=(65536) 22354
ph13=(65536) 25605
ph14=(65536) 11099
ph31= 1 3 3 1

;RF PULSES
; p11 : f1 channel – power level for pulse (default, normally 2.65 dB)
; p1 : f1 channel – 90 degree high power pulse (about 8-9 us)
; p18 : f1 channel – power level for binomial-like pulses
; p28 : f1 channel – 90 degree pulse at p18 (about 21 us)

; DELAYS
; d1 : relaxation delay; 1-5 * T1
; d16: delay for gradient recovery (100 us)
; d19: inter-pulse delay for binomial-like sequences
; d19 = (1/(2*d)), d = the bandwidth between two null points (in Hz)

; GRADIENTS
; p16: excitation sculpting gradient pulse (2 ms)
; gpz1: 34%
; gpz2: 22%
; NOTES
; NS: 8 * n
; DS: 4  (set to 0 if d1 allows full relaxation)
Excitation sculpting using PM2

; Excitation sculpting using PM2
; 1D sequence

#include <Avance.incl>
#include <Grad.incl>

1 ze
30u
2 d1 p11:f1
   p1 ph1
d16 UNBLKGRAD
p16:gp1
d16 p118:f1
p28*0.999924280 ph3
d19*2
p28*0.467998853 ph4
d19*2
p28*1.333333333 ph5
d19*2
p28*1.333333333 ph6
d19*2
p28*0.467998853 ph7
d19*2
p28*0.999924280 ph8
d16
p16:gp1
d16
d16
p16:gp2
d16
p28*0.999924280 ph9
d19*2
p28*0.467998853 ph10
d19*2
p28*1.333333333 ph11
d19*2
p28*1.333333333 ph12
d19*2
p28*0.467998853 ph13
d19*2
p28*0.999924280 ph14
d16
p16:gp2
d16
4u BLKGRAD
go=2 ph31
wr #0
exit

ph1=0 2
ph3=(65536) 39053 39053 55437 55437 6285 6285 22669 22669
ph4=(65536) 55434 55434 6282 6282 22666 22666 39050 39050
ph5=(65536) 55438 55438 6286 6286 22670 22670 39054 39054
ph6=(65536) 22670 22670 39054 39054 55438 55438 6286 6286
ph7=(65536) 22666 22666 39050 39050 55434 55434 6282 6282
ph8=(65536) 6285 6285 22669 22669 39053 39053 55437 55437
ph9=(65536) 39053
ph10=(65536) 55434
ph11=(65536) 55438
ph12=(65536) 22670
ph13=(65536) 22666
ph14=(65536) 6285
ph31= 1 3 3 1

;RF PULSES
; pl1 : f1 channel - power level for pulse (normally 2.65 dB)
; p1 : f1 channel - 90 degree high power pulse (about 8-9 us)
; p18: f1 channel - power level for binomial-like sequences
; p28: f1 channel - 90 degree pulse at p18 (about 21 us)

;DELAys
; d1 : relaxation delay; 1-5 * T1
; d16: delay for gradient recovery (100 us)
; d19: inter-pulse delay for binomial-like sequences
; d19 = (1/(2*d)), d = the bandwidth between two null points (in Hz)

;GRADIENTS
; p16: excitation sculpting gradient pulse (2 ms)
; gpz1: 34%
; gpz2: 22%

;NOTES
; NS: 8 * n
; DS: 4 (set to 0 if d1 allows full relaxation)
PGSTE-WATERGATE with PM1

; PGSTE-WATERGATE with PM1
; for DMX 500
; locked version

#include <Avance.incl>
#include <Grad.incl>

"p2  = p1 * 2"
"p20 = p16/2"

1 ze
30u
2 d1 p11:f1
p1 ph1
50u UNBLKGRAD
p20:gp1
d17 p118:f1
50u
p28*2.894120913 ph4
d19*2
p28*1.333333333 ph5
d19*2
p28*3.478208604 ph6
d19*2
p28*3.478208604 ph7
d19*2
p28*1.333333333 ph8
d19*2
p28*2.894120913 ph9
50u p11:f1
p20:gp3
d17
50u BLKGRAD
p1 ph2
50u UNBLKGRAD
p17:gp2
50u BLKGRAD
d25
p1 ph3
50u UNBLKGRAD
p20:gp3*-1
d17 p118:f1
50u
p28*2.894120913 ph10
d19*2
p28*1.333333333 ph11
d19*2
p28*3.478208604 ph12
d19*2
p28*3.478208604 ph13
d19*2
p28*1.333333333 ph14
d19*2
p28*2.894120913 ph15
  50u
p20:gp1*-1
d17
  50u BLKGRAD
go=2 ph31
  wr #0
  exit
ph1  = 0 2
ph2  = 0 1 2 3
ph3  = 0 1 2 3
ph4=(65536) 43867 43867 60251 60251 11099 11099 27483 27483
ph5=(65536) 58373 58373 9221 9221 25605 25605 41989 41989
ph6=(65536) 55122 55122 5970 5970 22354 22354 38738 38738
ph7=(65536) 22354 22354 38738 38738 55122 55122 5970 5970
ph8=(65536) 25605 25605 41989 41989 58373 58373 9221 9221
ph9=(65536) 11099 11099 27483 27483 43867 43867 60251 60251
ph10=(65536) 43867
ph11=(65536) 58373
ph12=(65536) 55122
ph13=(65536) 22354
ph14=(65536) 25605
ph15=(65536) 11099
ph31 = 1 3 3 1

; RF PULSES
; pl1 : f1 channel - power level for pulse (default) usually 2.65 dB
; pl18 : f1 channel - power level for binomial-like sequences
; pl1 : f1 channel - 90 degree high power pulse 8-9 us

; DELAYS
; d1 : relaxation delay 1-5 * T1
; d17 : gradient recovery delay (~ 100 us should be enough for high resolution probe)

; GRADIENTS
; p16: diffusion gradient pulse [1-8 ms]
;      gradient shape is controlled by gpnam1
;      gradient amplitude is controlled by gpz1
; p17: purge gradient pulse = typically [1-2 ms]
;      gradient shape is controlled by gpnam2
;      gradient amplitude is controlled by gpz2

; SCANS AND PHASE CYCLES
; NS: preferably 8 * n
; use the au program wp_diffamp to increment the gradients

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PGSTE-WATERGATE with PM2

; PGSTE-WATERGATE with PM2
; locked version

#include <Avance.incl>
#include <Grad.incl>

"p2 = p1 * 2"
"p20 = p16/2"

1 ze
  30u
2 d1 p11:f1
  p1 ph1
    50u UNBLKGRAD
    p20:gp1
    d17 p118:f1
    50u
    p28*0.999924280 ph4
d19*2
    p28*0.467998853 ph5
d19*2
    p28*1.333333333 ph6
d19*2
    p28*1.333333333 ph7
d19*2
    p28*0.467998853 ph8
d19*2
    p28*0.999924280 ph9
      50u p11:f1
      p20:gp3
      d17
      50u BLKGRAD
p1 ph2
  50u UNBLKGRAD
  p17:gp2
  50u BLKGRAD
d25
p1 ph3
  50u UNBLKGRAD
  p20:gp3*-1
  d17 p118:f1
  50u
  p28*0.999924280 ph10
d19*2
  p28*0.467998853 ph11
d19*2
  p28*1.333333333 ph12
d19*2
  p28*1.333333333 ph13
d19*2
  p28*0.467998853 ph14
d19*2
p28*0.999924280 ph15
  50u
p20:gp1*-1
d17  
  50u BLKGRAD
  go=2 ph31
  wr #0
exit

ph1  = 0 2
ph2  = 0 1 2 3
ph3  = 0 1 2 3
ph4=(65536) 39053 39053 55437 55437 6285 6285 22669 22669
ph5=(65536) 55434 55434 6282 6282 22666 22666 39050 39050
ph6=(65536) 55438 55438 6286 6286 22670 22670 39054 39054
ph7=(65536) 22670 22670 39054 39054 55438 55438 6286 6286
ph8=(65536) 22666 22666 39050 39050 55434 55434 6282 6282
ph9=(65536) 6285 6285 22669 22669 39053 39053 55437 55437
ph10=(65536) 39053
ph11=(65536) 55434
ph12=(65536) 55438
ph13=(65536) 22670
ph14=(65536) 22666
ph15=(65536) 6285
ph31 = 1 3 3 1

;RF PULSES
;p11 : f1 channel - power level for pulse (default) usually 2.65 dB
;p18 : f1 channel - power level for binominal-like sequences
;p1 : f1 channel - 90 degree high power pulse 8-9 us

;DELAYS
;d1  : relaxation delay 1-5 * T1
;d17 : gradient recovery delay (~ 100 us should be enough for high resolution probe)

;GRADIENTS
;p16: diffusion gradient pulse [1-8 ms]
;  gradient shape is controlled by gpnam1
;  gradient amplitude is controlled by gpz1
;p17: purge gradient pulse [1-2 ms]
;  gradient shape is controlled by gpnam2 (use wp_squa50)
;  gradient amplitude is controlled by gpz2

;SCANS AND PHASE CYCLES
;NS: preferably 8 * n
;use the au program wp_diffamp to increment the gradients
Asym-Max-PGSTE

; Asym-Max-PGSTE

; $CLASS=HighRes
; $DIM=1D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p2=p1*2"
"p4=p3*2"
"p20=p16/2"
"d2=1s/(cnst2*2)"
"d12=20u"
"DELTA=p1*4/3.1416"

1 ze
2 30m do:f2
d1
d12 p12:f2
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
50u UNBLKGRAMP
p20:gp1
d17
50u
(p2 ph2) (p4 ph1):f2
50u
p20:gp1*-1
50u BLKGRAMP
d17
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
50u UNBLKGRAMP
p17:gp2
d25
p17:gp2
d25
p17:gp2
50u BLKGRAMP
d17
(p1 ph1)
50u UNBLKGRAMP
p20:gp3
d17
50u
(p2 ph2)
50u
p20:gp3*-1
50u BLKGRAMP
d17
d12 pl12:f2
go=2 ph31 cpd2:f2
30m do:f2 mc #0 to 2 F0(zd)
exit

ph1=0
ph2=1
ph31=1

;p11 : f1 channel - power level for high power pulse (default)
p12 : f2 channel - power level for high power pulse (default)
p112: f2 channel - power level for CPD/BB decoupling
p1 : f1 channel - 90 degree high power pulse
p2 : f1 channel - 180 degree high power pulse
p3 : f2 channel - 90 degree high power pulse
p4 : f2 channel - 180 degree high power pulse
d1 : relaxation delay; 1-5 * T1
d2 : 1/(2J(XH))
d12: delay for power switching [20 usec]
cnst2: = J(XH)

;p16: diffusion encoding gradients
p17: purge gradients
g1/g3: = 4/13 4/9 and 4/5 for CH3 CH2 and CH respectively

;DS: 8
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;DELTA: delay to compensate for chemical shift evolution during
RF pulse in order to give same phase correction as
for reference 1D spectrum
Sym-Max-PGSTE

;Sym-Max-PGSTE

;CLASS=HighRes
;DIM=1D
;TYPE= 
;SUBTYPE= 
;COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p2=p1*2"
"p4=p3*2"
"p20=p16/2"
"d2=1s/(cnst2*2)"
"d12=20u"
"d18=p18"
"d19=1s/(cnst2*2) - 100u - p18 - d12"
"DELTA=p1*4/3.1416"

1 ze
2 30m do:f2
d1
d12 p12:f2
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
50u UNBLKGRAMP
p20:gp1
d17
50u
(p2 ph2) (p4 ph1):f2
50u
p20:gp1*-1
50u BLKGRAMP
d17
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
50u UNBLKGRAMP
p17:gp2
d25
p17:gp2
d25
p17:gp2
50u BLKGRAMP
d17
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
50u UNBLKGRAMP
p20:gp1
d18
d17
100u
(p2 ph2) (p4 ph1):f2
50u
p20:gp1*-1
50u
p18:gp3
50u BLKGRAMP
d17
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
50u
p20:gp1*1
50u
p18:gp3
50u BLKGRAMP
d19
d12 pl12:f2
go=2 ph31 cpd2:f2
30m do:f2 mc #0 to 2 F0(zd)
exit
ph1=0
ph2=1
ph31=1

; p11 : f1 channel - power level for high power pulse (default)
; p12 : f2 channel - power level for high power pulse (default)
; pl12: f2 channel - power level for CPD/BB decoupling
; pl : f1 channel - 90 degree high power pulse
; p2 : f1 channel - 180 degree high power pulse
; p3 : f2 channel - 90 degree high power pulse
; p4 : f2 channel - 180 degree high power pulse
; d1 : relaxation delay; 1-5 * T1
; d2 : 1/(2J(XH))
; d12: delay for power switching [20 usec]
; cnst2: = J(XH)

; gp1: diffusion encoding gradients
; gp2: purge gradients
; gp3, gp4: MQ coherence selection gradients
; gp3/gp4: = 4/13 4/9 and 4/5 for CH3 CH2 and CH respectively

; DS: 8
; cpd2: decoupling according to sequence defined by cpdprg2
; pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;DELTA: delay to compensate for chemical shift evolution during
; RF pulse in order to give same phase correction as
; for reference 1D spectrum
Max-SpinOrder-PGSTE

;Max-SpinOrder-PGSTE

;CLASS=HighRes
;DIM=1D
;TYPE=
;SUBTYPE=
;COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p2=p1*2"
"p4=p3*2"
"p20=p16/2"
"d2=1s/(cnst2*2)"
"d12=20u"
"d18=p18"
"d20=1s/(cnst2*2) - 50u - p18 - 50u - d12"
"DELTA=p1*4/3.1416"

1 ze
2 30m do:f2
d1
  d12 p12:f2
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
  50u UNBLKGRAMP
  p20:gp1
d17
  50u
(p2 ph2) (p4 ph1):f2
  50u
  p20:gp1*-1
  50u BLKGRAMP
d17
(p1 ph1) (p3 ph1):f2
  50u UNBLKGRAMP
  p17:gp2
d25
  p17:gp2
d25
  p17:gp2
  50u BLKGRAMP
d17
(p1 ph1) (p3 ph1):f2
  50u UNBLKGRAMP
  p20:gp1
  50u
d18
50u
d17
(p2 ph2) (p4 ph1):f2
50u
p20:gp1*-1
50u
p18:gp3
50u BLKGRAMP
d17
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
50u UNBLKGRAMP
p18:gp4
50u BLKGRAMP
d20
d12 pl12:f2
go=2 ph31 cpd2:f2
30m do:f2 mc #0 to 2 F0(zd)
exit

ph1=0
ph2=1
ph31=1

;p11 : f1 channel - power level for high power pulse (default)
p12 : f2 channel - power level for high power pulse (default)
p112: f2 channel - power level for CPD/BB decoupling
p1 : f1 channel - 90 degree high power pulse
p2 : f1 channel - 180 degree high power pulse
p3 : f2 channel - 90 degree high power pulse
p4 : f2 channel - 180 degree high power pulse
d1 : relaxation delay; 1-5 * T1
d2 : 1/(2J(XH))
d12: delay for power switching [20 usec]
cnst2: = J(XH)

;gp1: diffusion encoding gradients
gp2: purge gradients
;gp3, gp4: MQ coherence selection gradients
; gp3/gp4: = 4/13 4/9 and 4/5 for CH3 CH2 and CH respectively

;DS: 8
cpd2: decoupling according to sequence defined by cpdprg2
cpd2: f2 channel - 90 degree pulse for decoupling sequence

;DELTA: delay to compensate for chemical shift evolution during RF pulse in order to give same phase correction as for reference 1D spectrum
Asym-Anti-PGSTE

;Asym-Anti-PGSTE

;CLASS=HighRes
;DIM=1D
;TYPE=
;SUBTYPE=
;COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p2=p1*2"
"p4=p3*2"
"p20=p16/2"
"d2=1s/(cnst2*2)"
"d12=20u"
"d18=1s/(cnst2*2) - 50u - 50u - p20 - p1*1.5"

"DELTA=p1*4/3.1416"

1 ze
2 30m do:f2
d1
d12 p12:f2
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1) (p3 ph1):f2
50u UNBLKGRAMP
p20:gp1
d18
50u
(p2 ph2) (p4 ph1):f2
50u
p20:gp1*-1
50u BLKGRAMP
d18
(p1 ph1) (p3 ph1):f2
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1)
50u UNBLKGRAMP
p17:gp2
d25
p17:gp2
d25
p17:gp2
50u BLKGRAMP
d17
(p1 ph1)
  50u UNBLKGRAMP
  p20:gp3
d17
  50u
(p2 ph2)
  50u
  p20:gp3*-1
  50u BLKGRAMP
d17
d12 pl12:f2
go=2 ph31 cpd2:f2
  30m do:f2 mc #0 to 2 F0(zd)
exit

ph1=0
ph2=1
ph31=1

;pl1 : f1 channel - power level for high power pulse (default)
;pl2 : f2 channel - power level for high power pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel - 90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/(2J(XH))
;d12: delay for power switching [20 usec]
;cnst2: = J(XH)

;gp1,gp3: diffusion encoding gradients
;gp2: purge gradients
;gp1/gp3: = 4/13 4/9 and 4/5 for CH3 CH2 and CH respectively

;DS: 8
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;DELTA: delay to compensate for chemical shift evolution during
;        RF pulse in order to give same phase correction as
;        for reference 1D spectrum
Anti-SpinOrder-PGSTE

;Anti-SpinOrder-PGSTE

;CLASS=HighRes
;DIM=1D
;TYPE=
;SUBTYPE=
;COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p2=p1*2"
"p4=p3*2"
"p20=p16/2"
"d2=ls/(cnst2*2)"
"d12=20u"
"d18=ls/(cnst2*2) - 50u - p20 - 50u"
"d19=ls/(cnst2*2) - 50u - p20 - 50u - p18 - 50u"
"d20=ls/(cnst2*2) - 50u - p18 - 50u - d12"
"DELTA=p1*4/3.1416"

1 ze
2 30m do:f2
d1
d12 p12:f2
(p1 ph1)
d2
(p2 ph1) (p3 ph1):f2
d2
(p1 ph1) (p3 ph1):f2
50u UNBLKGRAMP
p20:gp1
d18
50u
(p2 ph2)(p4 ph1):f2
50u
p20:gp1*-1
50u BLKGRAMP
d18
(p1 ph1)
50u UNBLKGRAMP
p17:gp2
d25
p17:gp2
d25
p17:gp2
50u BLKGRAMP
d17
(p1 ph1)
50u UNBLKGRAMP
p20:gp1
d18
50u
(p2 ph2) (p4 ph1):f2
50u
p20:gp1*-1
50u
p18:gp3
50u BLKGRAMP
d19
(p1 ph1) (p3 ph1):f2
d2
(p2 ph1) (p3 ph1):f2
50u UNBLKGRAMP
p18:gp4
50u BLKGRAMP
d20
d12 pl12:f2
go=2 ph31 cpd2:f2
30m do:f2 mc #0 to 2 F0(zd)
exit

ph1=0
ph2=1
ph31=1

; pl1 : f1 channel - power level for high power pulse (default)
; pl2 : f2 channel - power level for high power pulse (default)
; pl12: f2 channel - power level for CPD/BB decoupling
; p1 : f1 channel - 90 degree high power pulse
; p2 : f1 channel - 180 degree high power pulse
; p3 : f2 channel - 90 degree high power pulse
; p4 : f2 channel - 180 degree high power pulse
; d1 : relaxation delay; 1-5 * T1
; d2 : 1/(2J(XH))
; d12: delay for power switching [20 usec]
; cnst2: = J(XH)

; gp1: diffusion encoding gradients
; gp2: purge gradients
; gp3, gp4: MQ coherence selection gradients
; gp3/gp4: = 4/13 4/9 and 4/5 for CH3 CH2 and CH respectively

; DS: 8
; cpd2: decoupling according to sequence defined by cpdprg2
; pcpd2: f2 channel - 90 degree pulse for decoupling sequence

; DELTA: delay to compensate for chemical shift evolution during
; RF pulse in order to give same phase correction as
; for reference 1D spectrum
APPENDIX B Maple Codes

Stejskal–Tanner analysis of the MAG-PGSTE sequence (with rectangular gradients)


Define the integral used in determining F

\[ F := (G, t_i) \rightarrow \int_{t_i}^{t_f} G \, dt \]

Define the time intervals and the relevant value of G for each integral. Also calculate the value of F for each interval remembering that it contains the contribution from all of the intervals from the start of the pulse sequence.

\begin{align*}
> l1 &:= 0; \\
> g1 &:= -gb1; \\
> F1 &:= F(g1, l1); \\
> l2 &:= \tau 1; \\
> g2 &:= gb1; \\
> F2 &:= \text{subs}(tf=l2, F1)+F(g2, l2); \\
> l3 &:= \tau 1+\delta 1; \\
> g3 &:= ga1+gb1; \\
> F3 &:= \text{subs}(tf=l3, F2)+F(g3, l3); \\
> l4 &:= \tau 1+\delta 1+\delta \\
> g4 &:= gb1; \\
> F4 &:= \text{subs}(tf=l4, F3)+F(g4, l4); \\
> l5 &:= 3*\tau 1;
\end{align*}
\[ g_5 := -gb_1; \]
\[ F_5 := -gb_1 \tau_1 + gb_1 \delta_1 + ga_1 \delta + gb_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 (\tau f - 3 \tau_1) \]
\[ l_6 := 3 \tau_1 + \delta_3 \]
\[ g_6 := -gb_1 - ga_2; \]
\[ F_6 := -gb_1 \tau_1 + gb_1 \delta + ga_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - ga_2 \delta \]
\[ l_7 := 3 \tau_1 + \delta_3 + \delta \]
\[ g_7 := -gb_1; \]
\[ F_7 := -gb_1 \tau_1 + gb_1 \delta + ga_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - ga_2 \delta \]
\[ l_8 := 5 \tau_1 \]
\[ g_8 := gb_1; \]
\[ F_8 := -gb_1 \tau_1 + gb_1 \delta + ga_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - ga_2 \delta - gb_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ l_9 := 6 \tau_1 \]
\[ g_9 := 0; \]
\[ F_9 := -gb_1 \tau_1 + gb_1 \delta + ga_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - ga_2 \delta - gb_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ l_{10} := 6 \tau_1 + \tau_2; \]
\[ g_{10} := gb_2; \]
\[ F_{10} := -gb_1 \tau_1 + gb_1 \delta + ga_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - ga_2 \delta - gb_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ l_{11} := 7 \tau_1 + \tau_2; \]
\[ g_{11} := -gb_2; \]
\[ F_{11} := -gb_1 \tau_1 + gb_1 \delta + ga_1 \delta + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - ga_2 \delta - gb_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ l_{12} := 7 \tau_1 + \tau_2 + \delta_1; \]
\[ g_{12} := ga_2 - gb_2; \]
> l13:=7*tau1+tau2+delta1+delta;
> g13:=-gb2;
> l13 := 7 \tau 1 + \tau 2 + \delta 1 + \delta
> g13 := -gb2
> F13:=subs(tf=l13,F12)+F(g13,l13);
> F13 := gb1 \delta 1 + ga1 \delta + gb1 (2 \tau 1 - \delta 1 - \delta) - gb1 \delta 3 - gb1 (2 \tau 1 - \delta 3 - \delta) + gb2 \tau 1 - gb2 \delta - gb2 (tf - 7 \tau 1 - \tau 2 - \delta 1 - \delta)
> l14:=tau2+9*tau1;
> l14 := \tau 2 + 9 \tau 1
> g14:=gb2;
> g14 := gb2
> F14:=subs(tf=l14,F13)+F(g14,l14);
> F14 := gb1 \delta 1 + ga1 \delta + gb1 (2 \tau 1 - \delta 1 - \delta) - gb1 \delta 3 - gb1 (2 \tau 1 - \delta 3 - \delta) + gb2 \tau 1 - gb2 \delta - gb2 (2 \tau 1 - \delta 1 - \delta) + gb2 (tf - \tau 2 - 9 \tau 1)
> l15:=tau2+9*tau1+delta3;
> l15 := \tau 2 + 9 \tau 1 + \delta 3
> g15:=gb2-ga1;
> g15 := gb2 - ga1
> F15:=subs(tf=l15,F14)+F(g15,l15);
> F15 := gb1 \delta 1 + ga1 \delta + gb1 (2 \tau 1 - \delta 1 - \delta) - gb1 \delta 3 - gb1 (2 \tau 1 - \delta 3 - \delta) + gb2 \tau 1 - gb2 \delta - gb2 (2 \tau 1 - \delta 1 - \delta) + gb2 \delta 3 + gb2 (tf - \tau 2 - 9 \tau 1 - \delta 3)
> l16:=tau2+9*tau1+delta3+delta;
> l16 := \tau 2 + 9 \tau 1 + \delta 3 + \delta
> g16:=gb2;
> g16 := gb2
> F16:=subs(tf=l16,F15)+F(g16,l16);
> F16 := gb1 \delta 1 + gb1 (2 \tau 1 - \delta 1 - \delta) - gb1 \delta 3 - gb1 (2 \tau 1 - \delta 3 - \delta) + gb2 \tau 1 - gb2 \delta 1 - gb2 (2 \tau 1 - \delta 1 - \delta) + gb2 \delta 3 + gb2 (tf - \tau 2 - 9 \tau 1 - \delta 3)
> l17:=11*tau1+tau2;
> l17 := 11 \tau 1 + \tau 2
> g17:=-gb2;
> g17 := -gb2
> F17:=subs(tf=l17,F16)+F(g17,l17);
> F17 := gb1 \delta 1 + gb1 (2 \tau 1 - \delta 1 - \delta) - gb1 \delta 3 - gb1 (2 \tau 1 - \delta 3 - \delta) + gb2 \tau 1 - gb2 \delta 1 - gb2 (2 \tau 1 - \delta 1 - \delta) + gb2 \delta 3 + gb2 (2 \tau 1 - \delta 3 - \delta) - gb2 (tf - 11 \tau 1 - \tau 2)
> l18:=tau2+12*tau1;
> l18 := \tau 2 + 12 \tau 1
> F1:=simplify(F1);
> F1 := -gb1 \text{if}
> F2:=simplify(F2);
> F2 := -2 gb1 \tau 1 + gb1 \text{if}
> F3:=simplify(F3);
> F3 := -2 gb1 \tau 1 + ga1 \text{if} - ga1 \tau 1 - ga1 \delta 1 + gb1 \text{if}
> F4:=simplify(F4);
> F4 := -2 gb1 \tau 1 + ga1 \delta + gb1 \text{if}
> F5:=simplify(F5);
> F5 := 4 gb1 \tau 1 + ga1 \delta - gb1 \text{if}
> F6:=simplify(F6);
> F6 := 4 gb1 \tau 1 + ga1 \delta - gb1 \text{if} - ga2 \tau 1 + 3 ga2 \tau 1 + ga2 \delta 3
> F7:=simplify(F7);
> F7 := 4 gb1 \tau 1 + ga1 \delta - ga2 \delta - gb1 \text{if}
\begin{align*}
F8 &:= \text{simplify}(F8) ; \\
F8 &:= -6 \, gb1 \, \tau 1 + gal \, \delta - ga2 \, \delta + gb1 \, tf \\
F9 &:= \text{simplify}(F9) ; \\
F9 &:= gal \, \delta - ga2 \, \delta \\
F10 &:= \text{simplify}(F10) ; \\
F10 &:= \text{collect}(%, gb1) ; \\
F11 &:= \text{simplify}(F11) ; \\
F11 &:= \text{collect}(%, gb1) ; \\
F12 &:= \text{simplify}(F12) ; \\
F12 &:= gal \, \delta - ga2 \, \delta + 8 \, gb2 \, \tau 1 - gb2 \, tf + gb2 \, \tau 2 \\
F13 &:= \text{simplify}(F13) ; \\
F13 &:= gal \, \delta + 8 \, gb2 \, \tau 1 - gb2 \, tf + gb2 \, \tau 2 \\
F14 &:= \text{simplify}(F14) ; \\
F14 &:= gal \, \delta - 10 \, gb2 \, \tau 1 + gb2 \, tf - gb2 \, \tau 2 \\
F15 &:= \text{simplify}(F15) ; \\
F15 &:= \text{collect}(%, gb1) ; \\
F16 &:= \text{simplify}(F16) ; \\
F16 &:= -10 \, gb2 \, \tau 1 + gb2 \, tf - gb2 \, \tau 2 \\
F17 &:= \text{simplify}(F17) ; \\
F17 &:= 12 \, gb2 \, \tau 1 - gb2 \, tf + gb2 \, \tau 2 \\
\end{align*}

The attenuation at the end of the encoding interval

\[ \ln(E1) := -\gamma^2 \, D \left(6 \, gb1^2 \, \tau^1 - gb1 \, gal \, \delta^3 + 6 \, gb1 \, \tau \, \delta^3 \, ga2 + 6 \, gb1 \, \tau \, \delta \, gal \, \delta \right) \]

\[ \begin{align*}
-3 \, gb1 \, \delta^1 \, gal \, \delta^2 - 3 \, gb1 \, \delta^3 \, ga2 + 3 \, gb1 \, \tau \, gal \, \delta^2 - 3 \, gb1 \, \delta^1 \, gal \, \delta \\
-3 \, gb1 \, \tau^1 \, gal \, \delta - gb1 \, \delta^3 \, ga2 + 3 \, gb1 \, \tau \, \delta^2 \, ga2 + 3 \, gb1 \, \tau \, \delta^2 \, ga2 \\
-3 \, gb1 \, \delta^3 \, ga2 - 18 \, \tau \, gal \, \delta^3 \, ga2 + 6 \, \delta^3 \, ga2 \, gal - 2 \, ga2^2 \, \delta^3 - 2 \, \delta^3 \, ga2^2 \\
+ 15 \, \tau \, gal^2 \, \delta^2 - 3 \, \delta^1 \, gal^2 \, \delta^2 + 3 \, \delta^3 \, ga2 + 9 \, \tau \, ga2^2 \, \delta^2 - 3 \, \delta^3 \, ga2^2 \, \delta^2)/3
\end{align*} \]

Cross-term

\[ \gamma^2 \, D \, \delta \, (gal \, \delta^2 \, gb1 - 6 \, gb1 \, \tau \, \delta \, ga2 - 6 \, gb1 \, \tau \, \delta \, gal + 3 \, gb1 \, \delta \, gal \, \delta \\
+ 3 \, gb1 \, \delta \, ga2 \, \delta - 3 \, gb1 \, \tau \, gal + 3 \, gb1 \, \tau^2 \, gal + 3 \, gb1 \, \delta^2 \, ga2 \\
- 3 \, gb1 \, \tau^2 \, ga2 - 3 \, gb1 \, \tau \, ga2 \, \delta + 3 \, gb1 \, \delta^3 \, ga2 + 18 \, \tau \, gal \, \delta \, ga2 \\
- 6 \, \delta^3 \, ga2 \, gal + 2 \, ga2^2 \, \delta^2 + 2 \, ga2^2 \, \delta^2 - 15 \, \tau \, gal^2 \, \delta + 3 \, \delta^1 \, gal^2 \, \delta \\
- 3 \, \delta^2 \, ga2^2 - 9 \, \tau \, ga2^2 \, \delta + 3 \, \delta^3 \, ga2^2 \, \delta)/3
\]

\[ \gamma^2 \, D \, \delta \, (gal \, \delta^2 - 6 \, \tau \, \delta \, ga2 - 6 \, \tau \, \delta \, gal + 3 \, \delta \, gal + 3 \, \delta^3 \, ga2 \, \delta - 3 \, \tau \, gal \, \delta \\
+ 3 \, \delta^2 \, gal + 3 \, \tau^2 \, gal + \delta^2 \, ga2 - 3 \, \tau^2 \, ga2 - 3 \, \tau \, gal \, \delta + 3 \, \delta^3 \, ga2) \, gb1/3 + \gamma^2 \, D \, \delta \, (-6 \, \delta^3 \, ga2 \, gal + 2 \, ga2^2 \, \delta^2 + 2 \, ga2^2 \, \delta^2 - 15 \, \tau \, gal^2 \, \delta + 3 \, \delta^1 \, gal^2 \, \delta \\
- 3 \, gal \, \delta^2 \, ga2^2 - 9 \, \tau \, gal^2 \, \delta + 3 \, \delta^3 \, ga2^2 \, \delta + 18 \, \tau \, gal \, \delta \, ga2)/3
\]

\[ \text{kkk} := \text{gal}^2 \, \delta^2 - 6 \, \tau \, gal \, \delta + 3 \, \delta \, gal + 3 \, \delta^3 \, ga2 + 3 \, \tau \, gal \, \delta + 3 \, \delta^3 \, ga2 \, gal + 2 \, ga2^2 \, \delta^2 + 2 \, ga2^2 \, \delta^2 - 15 \, \tau \, gal^2 \, \delta + 3 \, \delta^1 \, gal^2 \, \delta \\
- 3 \, gal \, \delta^2 \, ga2^2 - 9 \, \tau \, gal^2 \, \delta + 3 \, \delta^3 \, ga2^2 \, \delta + 18 \, \tau \, gal \, \delta \, ga2)/3
\]
The squared g0 term

> kk:=simplify(subs({ga2=0,ga1=0},ln(E)));  

The squared ga term

> kkl:=simplify(subs({gb1=0,gb2=0},ln(E)));
\[ \begin{align*}
kk1 &= -\gamma^2 D \delta^2 (3 \tau_2 \ga_1 \delta^2 + 3 \delta_3 \ga_1 \delta^2 - 24 \tau_1 \ga_1 \ga_2 + 6 \delta_3 \ga_2 \ga_1 - \ga_1 \delta^2 \delta - 3 \delta \ga_2^2 \\
&+ 24 \tau_1 \ga_1 \delta^2 - 3 \delta_1 \ga_1 \delta^2 + 12 \tau_1 \ga_2 \delta^2 - 3 \delta_3 \ga_2 \delta^2 + 3 \delta_2 \delta_1 + 3 \ga_2^2 \tau_2 \\
&- 6 \ga_1 \ga_2 \tau_2 - 6 \ga_1 \delta \ga_2) / 3
\end{align*} \]

\[
> \text{simplify(subs(Delta=tau2+6*tau1,kk1+gamma^2*D*delta^2*((Delta} + 2*tau1+delta-3-delta-*delta/3)*(ga1-ga2)^2+(4*tau1+2*(delta3-delta1)- 2*delta/3)*(ga2^2))));
\]

\[
0
\]

\[
> \text{simplify(subs((ga1=ga2+gg),kk1));}
\]

\[
-\gamma^2 D \delta^2 (-12 \ga_2 \delta_1 \gg + 12 \delta_3 \ga_2 \gg + 24 \tau_1 \ga_1 \gg + 24 \tau_1 \gg \delta^2 + 3 \delta_3 \gg^2 + 3 \ga_2^2 \tau_2 \\
&- 3 \delta_1 \gg + \delta \gg^2 + 2 \delta_1 \delta_2 \gg + 2 \delta_1 \gg^2 - 6 \delta_3 \delta_1 \ga_2^2 - 2 \delta \ga_2 \gg^2) / 3
\]

\[
> \text{simplify(subs((tau2=Deltat-6*tau1),%));}
\]

\[
-\gamma^2 D \delta^2 (-12 \ga_2 \delta_1 + 12 \delta_3 \ga_2 \delta_2 + 24 \ga_2 \tau_1 - 2 \ga_2 \delta) \gg
\]

\[
> \text{collect(%,gg);}
\]

\[
-\gamma^2 D \delta^2 (6 \tau_1 + 3 \Delta - 3 \delta_1 - 3 \delta_3) \gg^2
\]

\[
-\gamma^2 D \delta^2 (-12 \ga_2 \delta_1 + 12 \delta_3 \ga_2 \delta_2 + 24 \ga_2 \tau_1 - 2 \ga_2 \delta) \gg
\]

\[
-\gamma^2 D \delta^2 (12 \tau_1 \ga_2 \delta^2 + 6 \delta_3 \ga_2 \delta_2 - 6 \delta_1 \ga_2 \delta^2 - 2 \delta \ga_2 \gg^2)
\]

\[
> \text{collect(%,ga2);}
\]

\[
-\gamma^2 D \delta^2 (12 \tau_1 + 6 \delta_3 - 2 \delta) \ga_2^2
\]

\[
-\gamma^2 D \delta^2 (-12 \delta_1 + 12 \delta_3 + 24 \tau_1 - 2 \delta) \gg \ga_2
\]

\[
-\gamma^2 D \delta^2 (6 \tau_1 + 3 \Delta - 3 \delta_1 - \delta + 3 \delta_3) \gg^2
\]

The cross term

\[
> \text{kk2:=simplify(subs((gb1^2=0,gb2^2=0,ga1^2=0,ga2^2=0),ln(E)));}
\]

\[
kk2 := -\gamma^2 D \delta (gb1 \delta^2 \ga_2 - 3 \delta_1 \tau_1 \ga_1 \delta + 3 \delta_1 \delta_3 \ga_2 \delta - 3 \delta_1 \ga_1 \delta \\
&+ 3 \ga_1 \delta \gb_1 \delta_1 - 3 \ga_2 \delta \gb_2 \delta_1 - \ga_1 \delta^2 \gb_2 + 24 \tau_1 \ga_1 \delta \ga_2 - 6 \delta_3 \delta_3 \ga_2 \ga_1 \\
&- 3 \delta_2 \ga_2 \gb_2 \tau_1 + 3 \delta_1 \delta_2 \gb_1 + 3 \delta_1 \gb_2 \tau_1 - 3 \delta_2 \gb_2 \gb_1 + 3 \tau_1 \ga_2 \gb_2 - 3 \delta_2 \gb_2 \gb_1 + 3 \delta_2 \gb_2 \ga_1 \\
&- 3 \delta_1 \ga_1 \gb_2 + 3 \delta_3 \delta_1 \ga_1 + 3 \gb_1 \tau_1 \ga_1 - 3 \gb_1 \ga_1 \ga_1 - 3 \gb_1 \delta_2 \ga_2 + 3 \delta_2 \ga_2 \gb_2 \\
&- 3 \delta_1 \gb_3 \gb_2 + 3 \tau_1 \gb_2 \gb_2 + 6 \ga_1 \ga_1 \gb_2 \gb_2 + 6 \ga_1 \ga_1 \gb_2 \gb_2 - 6 \ga_1 \ga_1 \gb_2 \gb_2 + 6 \ga_1 \gb_3 \gb_2 \gb_2)
\]

\[
> \text{kcl:=collect(subs(ga1=0,kk2),ga2);}
\]

\[
kcl := -\gamma^2 D \delta (3 \gb_1 \delta^2 + 3 \delta_3 \delta - 3 \delta \gb_2 \delta_1 + 3 \gb_2 \delta_1 - 3 \delta \gb_2 \tau_1 - 3 \delta \gb_2 \gb_2 \\
&- 3 \gb_1 \tau_1^2 + 3 \delta_1 \gb_2 \gb_2 - 3 \gb_1 \gb_2 \gb_2 - 6 \gb_1 \ga_1 \gb_3 + 6 \ga_1 \gb_3 \gb_2)
\]

\[
> \text{kc2:=collect(subs(ga2=0,kk2),ga1);}
\]

\[
kc2 := -\gamma^2 D \delta (-3 \gb_1 \tau_1 \delta + 3 \delta_1 \gb_1 \delta_1 - 3 \delta \gb_2 \gb_2 + 6 \gb_1 \delta_2 + 3 \delta_2 \gb_2 \tau_1 - 3 \delta \gb_2 \gb_3 \\
&- 3 \gb_2 \gb_2 + 3 \gb_1 \delta_1^2 + 3 \gb_1 \tau_1^2 - 3 \delta_1 \ga_1 \gb_2 - 6 \gb_1 \ga_1 \delta_3)
\]

\[
> \text{kc3:=simplify(subs(ga2=eta*ga1,kcl+kc2));}
\]
\[ k_c^3 := \frac{\gamma^2 D \delta^3 \eta \text{gal} \text{gb1}}{3} + \gamma^2 D \delta^2 \eta \text{gal} \text{gb1} \delta 3 - \gamma^2 D \delta^2 \eta \text{gal} \text{gb1} \tau 1 \\
- \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \delta 1 + \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \tau 1 - \frac{\gamma^2 D \delta^3 \eta \text{gal} \text{gb2}}{3} \\
- \gamma^2 D \delta \eta \text{gal} \text{gb1} \tau 1^2 + \gamma^2 D \delta \eta \text{gal} \text{gb1} \delta 3^2 - \gamma^2 D \delta \eta \text{gal} \text{gb1} \delta 1^2 \text{gb2} \\
+ \gamma^2 D \delta \eta \text{gal} \tau 1^2 \text{gb2} - 2 \gamma^2 D \delta \eta \text{gal} \text{gb1} \tau 1 \delta 3 + 2 \gamma^2 D \delta \eta \text{gal} \tau 1 \delta 1 \text{gb2} \\
- \gamma^2 D \delta^2 \text{gal} \text{gb1} \tau 1 + \gamma^2 D \delta^2 \text{gal} \text{gb1} \delta 3 1 - \frac{\gamma^2 D \delta^3 \text{gal} \text{gb2}}{3} + \frac{\gamma^2 D \delta^3 \text{gal} \text{gb1}}{3} \\
+ \gamma^2 D \delta^2 \text{gal} \text{gb2} \tau 1 - \gamma^2 D \delta^2 \text{gal} \text{gb2} \delta 3 - \gamma^2 D \delta \text{gal} \tau 1^2 \text{gb2} \\
+ \gamma^2 D \delta \text{gal} \text{gb1} \delta 1^2 + \gamma^2 D \delta \text{gal} \text{gb1} \tau 1^2 - \gamma^2 D \delta \text{gal} \delta 3^2 \text{gb2} \\
- 2 \gamma^2 D \delta \text{gal} \text{gb1} \tau 1 \delta 1 + 2 \gamma^2 D \delta \text{gal} \tau 1 \delta 3 \text{gb2} \]

> Cross3 := solve(Cross3, eta);

Cross3 := \( -\gamma^2 D \delta \eta \text{gal} \delta 1^2 \text{gb2} + \gamma^2 D \delta \eta \text{gal} \tau 1^2 \text{gb2} + \gamma^2 D \delta \eta \text{gal} \text{gb1} \delta 1^2 \\
- \gamma^2 D \delta \eta \text{gal} \text{gb1} \tau 1^2 + \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \tau 1 - \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \delta 1 \\
- \gamma^2 D \delta^2 \eta \text{gal} \text{gb1} \tau 1 + \gamma^2 D \delta^2 \eta \text{gal} \text{gb1} \delta 1 + \frac{\gamma^2 D \delta^3 \eta \text{gal} \text{gb1}}{3} \\
- \frac{\gamma^2 D \delta^3 \eta \text{gal} \text{gb2}}{3} + 2 \gamma^2 D \delta \eta \text{gal} \tau 1 \delta 1 \text{gb2} - 2 \gamma^2 D \delta \eta \text{gal} \tau 1 \delta 1 \text{gb1} \\
- \gamma^2 D \delta \text{gal} \delta 1^2 \text{gb2} + \gamma^2 D \delta \text{gal} \text{gb1} \delta 1^2 + \gamma^2 D \delta \text{gal} \text{gb1} \tau 1^2 + \frac{\gamma^2 D \delta^3 \text{gal} \text{gb1}}{3} \\
+ \gamma^2 D \delta^2 \text{gal} \text{gb2} \tau 1 - \gamma^2 D \delta^2 \text{gal} \text{gb2} \delta 3 - \gamma^2 D \delta \text{gal} \tau 1^2 \text{gb2} \\
+ \gamma^2 D \delta \text{gal} \text{gb1} \delta 1^2 + \gamma^2 D \delta \text{gal} \text{gb1} \tau 1^2 - \gamma^2 D \delta \text{gal} \delta 3^2 \text{gb2} \\
- 2 \gamma^2 D \delta \text{gal} \text{gb1} \tau 1 \delta 1 + 2 \gamma^2 D \delta \text{gal} \tau 1 \delta 3 \text{gb2} \)

Calculate magic ratio

> sol3 := solve(Cross3, eta);

\[ \text{sol3} := \frac{-6 \tau 1 \delta 1 + 3 \delta 1^2 + 3 \delta 1 \delta + 3 \delta^2 - 3 \tau 1 \delta + 3 \tau 1^2}{-3 \delta 1 \delta - 3 \delta 1^2 + 6 \tau 1 \delta 1 - 3 \delta 1 \delta + 3 \tau 1^2} \]

> Cross4 := subs(delta3=2*tau1-delta1-delta, Cross3);

Cross4 := \( -\gamma^2 D \delta \eta \text{gal} \delta 1^2 \text{gb2} + \gamma^2 D \delta \eta \text{gal} \tau 1^2 \text{gb2} \\
+ \gamma^2 D \delta \eta \text{gal} \text{gb1} (2 \tau 1 - \delta 1 - \delta)^2 - \gamma^2 D \delta \eta \text{gal} \text{gb1} \tau 1^2 + \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \tau 1 \\
- \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \delta 1 - \gamma^2 D \delta^2 \eta \text{gal} \text{gb2} \delta 1 - \gamma^2 D \delta \text{gal} \tau 1^2 \text{gb2} + \frac{\gamma^2 D \delta^3 \text{gal} \text{gb1}}{3} \\
- \frac{\gamma^2 D \delta^3 \text{gal} \text{gb2}}{3} + 2 \gamma^2 D \delta \eta \text{gal} \tau 1 \delta 1 \text{gb2} \\
- 2 \gamma^2 D \delta \eta \text{gal} \text{gb1} \tau 1 (2 \tau 1 - \delta 1 - \delta) - \frac{\gamma^2 D \delta^3 \text{gal} \text{gb2}}{3} - \gamma^2 D \delta^2 \text{gal} \text{gb1} \tau 1 \\
+ \gamma^2 D \delta^2 \text{gal} \text{gb1} \delta 1 - \gamma^2 D \delta \text{gal} \tau 1^2 \text{gb2} - \gamma^2 D \delta \text{gal} \text{gb1} (2 \tau 1 - \delta 1 - \delta)^2 \text{gb2} \\
+ \gamma^2 D \delta \text{gal} \text{gb1} \delta 1^2 + \gamma^2 D \delta \text{gal} \text{gb1} \tau 1^2 + \frac{\gamma^2 D \delta^3 \text{gal} \text{gb1}}{3} + \gamma^2 D \delta^2 \text{gal} \text{gb2} \tau 1 \\
- \gamma^2 D \delta^2 \text{gal} \text{gb2} (2 \tau 1 - \delta 1 - \delta) + 2 \gamma^2 D \delta \text{gal} \tau 1 (2 \tau 1 - \delta 1 - \delta) \text{gb2} \\
- 2 \gamma^2 D \delta \text{gal} \text{gb1} \tau 1 \delta 1 \)
Calculate magic ratio

\texttt{solv} := \texttt{solve(Cross4,eta)};

\texttt{sol4} := \frac{-6 \tau l \delta l + 3 \delta l^2 + 3 \delta l \delta + \delta^2 - 3 \tau l \delta + 3 \tau l^2}{-3 \delta l \delta - 3 \delta l^2 + 6 \tau l \delta l - \delta^2 + 3 \tau l \delta + 3 \tau l^2}

\texttt{simplify(sol3-sol4)};

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Stejskal-Tanner analysis of the MAG-PGSTE sequence  
(with half-sine gradients) 
G. Zheng, William S. Price  (07/02/2008)

Define the integral used in determining $\mathbf{F}$
\[ F := (G, t_i) \rightarrow \int_{t_i}^{t_f} G \, dt \]

Define the time intervals and the relevant value of $G$ for each integral. Also calculate the value of $F$ for each interval remembering that it contains the contribution from all of the intervals from the start of the pulse sequence.

> \( l1 := 0; \)
> \( g1 := -gb1; \)
> \( F1 := F(g1, l1); \)
> \( l2 := \tau_1; \)
> \( g2 := gb1; \)
> \( F2 := \text{subs}(tf=l2, F1) + F(g2, l2); \)
> \( l3 := \tau_1 + \delta_1; \)
> \( g3 := ga1 \sin(\pi(t - l3)/\delta) + gb1; \)
> \( F3 := \text{subs}(tf=l3, F2) + F(g3, l3); \)
> \( l4 := 3\tau_1; \)
> \( g4 := gb1; \)
> \( F4 := \text{subs}(tf=l4, F3) + F(g4, l4); \)
> \( l5 := 3\tau_1; \)
> \( g5 := -gb1; \)
> \( F5 := \text{subs}(tf=l5, F4) + F(g5, l5); \)

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> l6 := 3*t1 + delta3
> g6 := -gb1 - ga2*sin(Pi*(t-16)/delta);
> F6 := subs(tf=l6,F5)+F(g6,l6);
> F6 := -gb1*t1 + gb1*delta3 + 2*ga1*delta - gb1*pi*t1 - gb1*pi*delta1 + gb1*(t1 + delta1 + delta)*pi
> + gb1*(2*t1 - delta1 - delta) - gb1*delta3
> - 3*gb1*pi*t1 - gb1*pi*delta3 + ga2*delta + gb1*(t1 + delta3 + delta)*pi
> - gb1*(t - 3*t1 - delta3 - delta)
> l7 := 3*t1 + delta3 + delta
> g7 := -gb1;
> F7 := subs(tf=l7,F6)+F(g7,l7);
> F7 := -gb1*t1 + gb1*delta1 + 2*ga1*delta - gb1*pi*t1 - gb1*pi*delta1 + gb1*(t1 + delta1 + delta)*pi
> + gb1*(2*t1 - delta1 - delta) - gb1*delta3
> - 3*gb1*pi*t1 - gb1*pi*delta3 + ga2*delta + gb1*(t1 + delta3 + delta)*pi
> - ga2*delta*cos(p)
> l8 := 5*t1
> g8 := gb1;
> F8 := subs(tf=l8,F7)+F(g8,l8);
> F8 := -gb1*t1 + gb1*delta1 + 2*ga1*delta - gb1*pi*t1 - gb1*pi*delta1 + gb1*(t1 + delta1 + delta)*pi
> + gb1*(2*t1 - delta1 - delta) - gb1*delta3
> - 3*gb1*pi*t1 - gb1*pi*delta3 + ga2*delta + gb1*(t1 + delta3 + delta)*pi
> - gb1*(t - 5*t1)
> l9 := 6*t1
> g9 := 0;
> F9 := subs(tf=l9,F8)+F(g9,l9);
> F9 := gb1*delta1 + 2*ga1*delta - gb1*pi*t1 - gb1*pi*delta1 + gb1*(t1 + delta1 + delta)*pi
> + gb1*(2*t1 - delta1 - delta)
> - gb1*delta3 - 3*gb1*pi*t1 - gb1*pi*delta3 + ga2*delta + gb1*(t1 + delta3 + delta)*pi
> - gb1*(2*t1 - delta3 - delta)
> l10 := 6*t1 + tau2
> g10 := gb2;
> F10 := subs(tf=l10,F9)+F(g10,l10);
\[ F_{10} := gb1 \delta_1 + \frac{2g1 \delta - gb1 \pi \tau_1 - gb1 \pi \delta_1 + gb1 (\tau_1 + \delta_1 + \delta) \pi}{\pi} \]
\[ + gb1 (2 \tau_1 - \delta_1 - \delta) - gb1 \delta \]
\[ - \frac{-3 gb1 \pi \tau_1 - gb1 \pi \delta_3 + 2 ga2 \delta + gb1 (3 \tau_1 + \delta_3 + \delta) \pi}{\pi} - gb1 (2 \tau_1 - \delta_3 - \delta) \]
\[ + gb2 (t_1 - 6 \tau_1 - t_2) \]
\> l11 := 7*tau1 + tau2;
\> g11 := -gb2;
\> F11 := subs(tf=l11,F10)+F(g11,l11);
\[ l11 := 7 \tau_1 + \tau_2 \]
\[ g11 := -gb2 \]
\[ F11 := gb1 \delta_1 + \frac{2g1 \delta - gb1 \pi \tau_1 - gb1 \pi \delta_1 + gb1 (\tau_1 + \delta_1 + \delta) \pi}{\pi} \]
\[ + gb1 (2 \tau_1 - \delta_1 - \delta) - gb1 \delta \]
\[ - \frac{-3 gb1 \pi \tau_1 - gb1 \pi \delta_3 + 2 ga2 \delta + gb1 (3 \tau_1 + \delta_3 + \delta) \pi}{\pi} - gb1 (2 \tau_1 - \delta_3 - \delta) \]
\[ + gb2 (t_1 - gb2 (t_1 - 7 \tau_1 - t_2)) \]
\> l12 := 7*tau1 + tau2 + delta1;
\> g12 := ga2*sin(Pi*(t-112)/delta)-gb2;
\[ l12 := 7 \tau_1 + \tau_2 + \delta_1 \]
\[ g12 := ga2 \sin\left(\frac{\pi(\tau_1 - \tau_2 - t_1)}{\delta}\right) - gb2 \]
\> F12 := subs(tf=l12,F11)+F(g12,l12);
\> l13 := 7*tau1 + tau2 + delta1 + delta;
\> g13 := -gb2;
\[ l13 := 7 \tau_1 + \tau_2 + \delta_1 + \delta \]
\[ g13 := -gb2 \]
\> F13 := subs(tf=l13,F12)+F(g13,l13);
\> l14 := tau2 + 9*tau1;
\> g14 := gb2;
\[ l14 := \tau_2 + 9 \tau_1 \]
\[ g14 := gb2 \]
\> F14 := subs(tf=l14,F13)+F(g14,l14);
\[ F_{14} := g_1 \delta_1 + \frac{2 \pi \delta_1 - g_1 \pi \delta_1 + g_1 \pi \delta_1 + g_1 (\tau_1 + \delta_1 + \delta) \pi}{g_1 \pi (2 \tau_1 - \delta_1 - \delta) - g_1 \delta_3} \]
\[ -3 g_1 \pi \tau_1 - g_1 \pi \delta_3 + 2 g_2 \delta + g_1 (3 \tau_1 + \delta_3 + \delta) \pi - g_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ + g_2 \tau_1 - g_2 \delta_1 \]
\[ -2 g_2 \delta - g_2 \pi \delta_1 - 7 g_2 \pi \tau_1 - g_2 \pi \tau_2 + g_2 (7 \tau_1 + \tau_2 + \delta_1 + \delta) \pi \]
\[ - g_2 (2 \tau_1 - \delta_1 - \delta) + g_2 (\tau_2 - 9 \tau_1) \]

> 115 := \text{tau}2 + 9*\text{tau}1 + \text{delta}3; 
\[ l_{15} := \text{tau}2 + 9 \tau_1 + \delta_3 \]
> g_{15} := g_2 - g_1 * \text{sin}(\pi * (t - 115) / \text{delta}); 
\[ g_{15} := g_2 - g_1 \sin \left( \frac{\pi (t - \tau_2 - 9 \tau_1 - \delta_3)}{\delta} \right) \]
> F_{15} := \text{subs}(\text{tf} = 115, F_{14}) + F(g_{15}, l_{15}); 
\[ F_{15} := g_1 \delta_1 + \frac{2 \pi \delta_1 - g_1 \pi \delta_1 + g_1 \pi \delta_1 + g_1 (\tau_1 + \delta_1 + \delta) \pi}{g_1 \pi (2 \tau_1 - \delta_1 - \delta) - g_1 \delta_3} \]
\[ -3 g_1 \pi \tau_1 - g_1 \pi \delta_3 + 2 g_2 \delta + g_1 (3 \tau_1 + \delta_3 + \delta) \pi - g_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ + g_2 \tau_1 - g_2 \delta_1 \]
\[ -2 g_2 \delta - g_2 \pi \delta_1 - 7 g_2 \pi \tau_1 - g_2 \pi \tau_2 + g_2 (7 \tau_1 + \tau_2 + \delta_1 + \delta) \pi \]
\[ - g_2 (2 \tau_1 - \delta_1 - \delta) + g_2 (\tau_2 - 9 \tau_1 - \delta_3 - \delta) + \frac{-g_1 \delta - g_2 \pi \tau_2 - 9 g_2 \pi \tau_1 - g_2 \pi \delta_3 + g_2 (\tau_2 + 9 \tau_1)}{\pi} \]
\[ + g_2 (\tau_1 - 9 \tau_1 - \delta_3 - \delta) \]

> 116 := \text{tau}2 + 9*\text{tau}1 + \text{delta}3 + \text{delta}; 
\[ l_{16} := \text{tau}2 + 9 \tau_1 + \delta_3 + \delta \]
> g_{16} := g_2; 
\[ g_{16} := g_2 \]
> F_{16} := \text{subs}(\text{tf} = 116, F_{15}) + F(g_{16}, l_{16}); 
\[ F_{16} := g_1 \delta_1 + \frac{2 \pi \delta_1 - g_1 \pi \delta_1 + g_1 \pi \delta_1 + g_1 (\tau_1 + \delta_1 + \delta) \pi}{g_1 \pi (2 \tau_1 - \delta_1 - \delta) - g_1 \delta_3} \]
\[ -3 g_1 \pi \tau_1 - g_1 \pi \delta_3 + 2 g_2 \delta + g_1 (3 \tau_1 + \delta_3 + \delta) \pi - g_1 (2 \tau_1 - \delta_3 - \delta) \]
\[ + g_2 \tau_1 - g_2 \delta_1 \]
\[ -2 g_2 \delta - g_2 \pi \delta_1 - 7 g_2 \pi \tau_1 - g_2 \pi \tau_2 + g_2 (7 \tau_1 + \tau_2 + \delta_1 + \delta) \pi \]
\[ - g_2 (2 \tau_1 - \delta_1 - \delta) + g_2 (\tau_2 + 9 \tau_1 - \delta_3 - \delta) \]
\[ + g_2 (\tau_1 - 9 \tau_1 - \delta_3 - \delta) \]

> 117 := l_{11} * \text{tau}1 + \text{tau}2; 
\[ l_{17} := 11 \tau_1 + \tau_2 \]
> g_{17} := -g_2; 
\[ g_{17} := -g_2 \]
> F_{17} := \text{subs}(\text{tf} = 117, F_{16}) + F(g_{17}, l_{17});
\[ F_{17} := gb_1 \delta_1 + \frac{2 \text{gal} \delta - gb_1 \pi \tau_1 - gb_1 \pi \delta_1 + gb_1 (\tau_1 + \delta_1 + \delta) \pi}{\pi} + gb_1 (2 \tau_1 - \delta_1 - \delta) - gb_1 \delta_3 - 3 gb_1 \pi \tau_1 - gb_1 \pi \delta_3 + 2 \text{gal} \delta + gb_1 (3 \tau_1 + \delta_3 + \delta) \pi - gb_1 (2 \tau_1 - \delta_3 - \delta) + gb_2 \pi \tau_1 - gb_2 \pi \delta_1 - 2 \text{ga}_2 \delta - gb_2 \pi \delta_1 - 7 gb_2 \pi \tau_1 - gb_2 \pi \tau_2 + gb_2 (7 \tau_1 + \tau_2 + \delta_1 + \delta) \pi - gb_2 (2 \tau_1 - \delta_1 - \delta) + gb_2 \delta_3 - 2 gb_2 \delta - gb_2 \pi \tau_2 - 9 gb_2 \pi \tau_1 - gb_2 \pi \delta_3 + gb_2 (\tau_2 + 9 \tau_1 + \delta_3 + \delta) \pi + gb_2 (2 \tau_1 - \delta_3 - \delta) - gb_2 (5f - 11 \tau_1 - \tau_2) \]
\[
F12 := -\frac{-2gal \delta + ga2 \delta - 8 gb2 \pi \tau 1 - gb2 \pi \tau 2 + ga2 \delta \cos \left( \frac{\pi (tf - 7 \tau 1 - \tau 2 - \delta 1)}{\delta} \right)}{\pi}
\]

\[
F13 := \frac{-2gal \delta - 8 gb2 \pi \tau 1 + gb2 \pi \tau 2}{\pi}
\]

\[
F14 := \frac{2gal \delta - 10 gb2 \pi \tau 1 + gb2 \pi \tau 2}{\pi}
\]

\[
F15 := \frac{gal \delta - 10 gb2 \pi \tau 1 - gb2 \pi \tau 2 + gb2 \pi \tau 2 + 4gal \delta + gb2 \pi \tau 2}{\pi}
\]

\[
F16 := gb2 (\pi - 2 - 10 \tau 1 + t f)
\]

\[
F17 := -gb2 (\pi - 2 - 12 \tau 1 + t f)
\]

The attenuation at the end of the first encoding interval

\[
\ln(E1) := \gamma^2 D \left( 8 ga2 \delta^3 gb1 + 24 \pi ga2 \delta^2 \tau 1 - 8 \pi ga2 \delta^2 \delta 3 - 48 \pi ga1 \delta^2 ga2 \tau 1 + 16 \pi ga1 \delta^2 ga2 \delta 3 + 4 gb1 \delta^2 \tau 1^3 - 5 ga2 \delta^3 \pi - 2 gal \delta^3 gb1 \pi^2
\]

\[
- 4 gal \delta^2 gb1 \pi^2 \delta 1 + 4 gb1 \pi^2 \tau 1 + 8 gas \delta^2 gb1 + 40 \pi gal \delta^2 \tau 1 - 8 \pi gal \delta^2 \delta 1 - 4 gb1 \pi^2 \tau 1 - 8 gb1 \pi^2 \delta 1 - 4 gb1 \pi^2 \delta 1 - 4 gb1 \pi^2 \delta 1
\]

\[
- 2 ga2 \delta^3 gb1 \pi^2 + 8 gal \delta^3 ga2 \pi + 4 gb1 \pi^2 \delta 1^2 + 8 gb1 \pi^2 \delta 1^2 - 4 gb1 \pi^2 \delta 1^2
\]

\[
- 4 gb1 \pi^2 \delta 1^2 + 4 gb1 \pi^2 \tau 1 + 8 gb1 \pi^2 \delta 1^2 - 5 ga2 \delta^3 \pi) / (2 \pi^3)
\]

Cross-term

\[
simplify(subs(gb1^2=0, \ln(E1)))
\]

\[
\gamma^2 D \delta \left(-8 ga2 \delta^2 gb1 - 24 \pi ga2 \delta^2 \delta 1 + 8 \pi ga2 \delta^2 \delta 3 + 48 \pi ga1 \delta ga2 \tau 1
\]

\[
- 16 \pi ga1 \delta ga2 \delta 3 + 5 ga2 \delta^2 \pi + 2 ga2 \delta^2 gb1 \pi^2 + 4 gal \delta gb1 \pi^2 \delta 1
\]

\[
- 4 gb1 \pi^2 \tau 1 + 8 gal \delta^2 gb1 - 40 \pi gal \delta^2 \tau 1 + 8 \pi gal \delta^2 \delta 1
\]

\[
+ 4 gb1 \pi^2 \tau 1 - 8 gb1 \pi^2 \tau 1 + 4 gb1 \pi^2 \tau 1 + 2 ga2 \delta^2 gb1 \pi^2
\]

\[
- 8 gal \delta^2 ga2 \pi - 4 gb1 \pi^2 ga2 \tau 1^2 - 8 gb1 \pi^2 ga2 \tau 1 \delta 3 + 4 gb1 \pi^2 ga2 \delta 3^2
\]

\[
- 4 gb1 \pi^2 \tau 1 + 8 gb1 \pi^2 \delta 3 + 5 gb1 \delta^2 \pi) / (2 \pi^3)
\]

\[
simplify(\% \ast gb1)
\]

\[
\gamma^2 D \delta \left(-8 ga2 \delta^2 + 2 ga2 \delta^2 \pi + 4 gal \delta^2 \pi + 4 ga2 \delta^2 \pi - 8 \pi ga2 \tau 1 \delta 1 + 4 ga2 \delta^2 \delta 1^2 + 2 ga2 \delta^2 \delta 1^2 - 4 \pi^2 \tau 1 + 2 ga2 \delta^2 \tau 1^2 - 8 ga2 \tau 1 \delta 3
\]

\[
+ 4 \pi^2 \tau 1 + 4 ga2 \delta^2 \tau 1 + 2 ga2 \delta^2 \delta 3) gb1 / (2 \pi^3)
\]

\[
-16 \pi ga1 \delta ga2 \delta 3 - 24 \pi ga2 \delta \tau 1 + 8 \pi ga2 \delta \delta 3 + 48 \pi ga1 \delta ga2 \tau 1
\]

\[
- 8 gal \delta^2 ga2 \pi + 5 ga1 \delta^2 \pi - 40 \pi ga1 \delta \tau 1 + 8 \pi ga1 \delta \delta 1 + 5 ga2 \delta^2 \pi) / (2 \pi^3)
\]

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The total attenuation

\[ \ln(E) = -\gamma \, D \, (4 \, gb1 \, \pi^2 \, ga2 \, \delta \, \tau \, \delta3 - 2 \, gb1 \, \pi^2 \, ga2 \, \delta \, \delta3^2 + 4 \, gb2 \, \delta^3 \, gb1 \\
+ 16 \, \pi \, ga2^2 \, \delta^2 \, \tau \, \delta3 - 4 \, gb1 \, \pi \, ga2^2 \, \delta^3 \, \delta3 + 32 \, \pi \, ga2^2 \, \delta^3 \, \tau - 4 \, \pi \, ga1^2 \, \delta^2 \, \delta3 \\
- 2 \, gb1 \, \delta^2 \, gb1 \, \pi \, \delta3 - 2 \, gb1 \, \pi \, \delta3^2 + 2 \, gb2 \, \delta^3 \, gb2 \\
+ 2 \, gb2 \, \delta3 \, gb2 \, \pi \, \delta3 - 2 \, gb2 \, \delta^2 \, gb1 \, \pi \, \delta3 - gb1 \, \pi \, \delta3 \, gb1 \, \pi^2 - 4 \, gb2 \, \delta^3 \, gb2 \\
+ 2 \, gb1 \, \delta3 \, gb2 \, \pi \, \delta3 - gb3 \, \delta3 \, gb2 \, \pi \, \delta3 - gb3 \, \delta3 \, \pi + gb3 \, \delta3 \, gb2 \, \pi^2) \]

\[ \text{Calculate magic ratio} \]

\[ \text{soll} := \text{solve(Cross1, } \eta) \]

\[ \text{Calculate magic ratio} \]

\[ \text{sol2} := \text{solve(Cross2, } \eta) \]

\[ \text{collect(}2 \, \eta) \]

\[ \text{simplify(sol1-sol2)} \]

\[ 0 \]
\[ + gal \, \delta^3 \, gb2 \, \pi^2 - 32 \, \pi \, gal \, \delta^2 \, ga2 \, \tau1 + 8 \, \pi \, gal \, \delta^2 \, ga2 \, \delta3 - 2 \, gb1 \, \pi^2 \, gal \, \delta \, \tau1^2 \\
+ 4 \, gb1 \, \pi^2 \, gal \, \delta \, \tau1 \, \delta1 - 2 \, gb1 \, \pi^2 \, gal \, \delta \, \delta1^2 + 2 \, gb1 \, \pi^2 \, \tau1 \, gal \, \delta^2 + 4 \, gal \, \delta^3 \, gb1 \\
- 4 \, gal \, \delta^3 \, gb2 + 2 \, \pi^3 \, \tau^1 \, gb^2 - 2 \, gal \, \delta^2 \, gb2 \, \pi^2 \, \tau1 - 2 \, gb2 \, \delta^2 \, gb2 \, \pi^2 \, \tau1 \\
+ 2 \, gb2 \, \delta^2 \, gb^2 \, \pi^2 \, \delta1 + 2 \, gb2 \, \delta1^2 \, \pi^2 \, ga2 \, \delta + 4 \, \delta1 \, \pi \, ga2 \, \delta^2 + 2 \, gb2 \, \delta1^2 \, \delta^2 \\
+ 4 \, \delta^2 \, \tau2 \, \pi \, ga1^2 + 4 \, \delta^2 \, \tau2 \, \pi \, ga2^2 - ga2 \, \delta^3 \, gb1 \, \pi^2 + 2 \, gb1 \, \pi^2 \, ga2 \, \delta \, \tau1^2 \\
- 8 \, \delta^2 \, \tau2 \, \pi \, ga1 \, ga2 - 4 \, \pi^2 \, \delta3 \, gal \, \delta \, gb2 \, \tau1 + 2 \, gb2 \, \delta^2 \, gb^2 \, \pi^2 \, ga \, \delta \\
+ 2 \, \pi^2 \, \tau^1 \, ga \, \delta \, gb2 - 2 \, \pi^2 \, \tau^1 \, ga2 \, \delta \, gb2 - 8 \, \delta1 \, \pi \, ga \, \delta^2 \, ga2 \\
- 4 \, \delta1 \, \pi^2 \, ga2 \, \delta \, gb2 \, \tau1 + 2 \, gb1 \, \pi^2 \, \tau1 \, ga2 \, \delta^2 ) / \pi^3 \]

The squared g0 term
> \texttt{kk:=simplify(subs(\{ga2=0,gal=0\},ln(E))};}
\[ kk := -2 \, \gamma^2 \, D \, \tau^1 \, (gb1^2 + gb2^2) \]

The squared ga term
> \texttt{kk1:=simplify(subs(\{gb1=0,gb2=0\},ln(E))};}
\[ kk1 := -\gamma^2 \, D \, \delta^2 \, (16 \, \tau1 \, ga2^2 - 4 \, gb2 \, \delta3 + 32 \, \tau1 \, gal \, \delta1^2 - 4 \, \delta1 \, ga1 \, \delta1 - ga2 \, \delta - ga2 \, \delta \\
- 32 \, \tau1 \, gal \, ga2 + 8 \, ga1 \, ga2 \, \delta3 + 4 \, \delta1 \, ga2 \, \delta2 + 4 \, \delta3 \, ga1 \, \delta2 + 4 \, \tau2 \, ga1 \, \delta2 + 4 \, \tau2 \, ga2^2 \\
- 8 \, \tau2 \, ga1 \, ga2 - 8 \, \delta1 \, \delta1 \, ga2) / \pi^2 \]
> \texttt{collect(%{gg},kk1)};
\[ -\gamma^2 \, D \, \delta^2 \, (32 \, \tau1 \, ga2 \, gg - 16 \, \delta1 \, ga2 \, gg + 16 \, ga2 \, \delta3 \, gg - 2 \, \delta \, ga2 \, gg + 4 \, \tau2 \, gg^2 + 4 \, \delta3 \, gg^2 \\
- \delta \, gg^2 - 4 \, \delta1 \, gg^2 + 32 \, \tau1 \, gg^2 + 16 \, \tau1 \, ga2 \, gg + 8 \, ga2 \, \delta3 - 8 \, \delta1 \, ga2^2 - 2 \, ga2 \, \delta) / \pi^2 \]
> \texttt{collect(%{tau2=Delta-6*tau1},%)};
\[ -\gamma^2 \, D \, \delta^2 \, (32 \, \tau1 \, ga2 \, gg - 16 \, \delta1 \, ga2 \, gg + 16 \, ga2 \, \delta3 \, gg - 2 \, \delta \, ga2 \, gg + 4 \, \tau2 \, gg^2 + 4 \, \delta3 \, gg^2 \\
+ 4 \, \delta3 \, gg^2 - \delta \, gg^2 - 4 \, \delta1 \, gg^2 + 16 \, \tau1 \, ga2 \, gg + 8 \, ga2 \, \delta3 - 8 \, \delta1 \, ga2^2 - 2 \, ga2 \, \delta) / \pi^2 \]
> \texttt{collect(%{gg},ga2)};
\[ -\gamma^2 \, D \, \delta^2 \, (16 \, \delta1 \, ga2 \, gg + 16 \, \delta1 \, ga2 \, gg + 16 \, ga2 \, \delta3 \, gg - 2 \, \delta \, ga2 \, gg + 4 \, \tau2 \, gg^2 + 4 \, \delta3 \, gg^2 \\
- \delta \, gg^2 - 4 \, \delta1 \, gg^2 + 32 \, \tau1 \, gg^2 + 16 \, \tau1 \, ga2 \, gg + 8 \, ga2 \, \delta3 - 8 \, \delta1 \, ga2^2 - 2 \, ga2 \, \delta) / \pi^2 \]
> \texttt{collect(%*(-Pi^2),ga2)};
\[ -\gamma^2 \, D \, \delta^2 \, (32 \, \tau1 \, ga2 \, gg + 8 \, ga2 \, \delta3 - 8 \, \delta1 \, ga2^2) \]
> \texttt{The cross term}
kk2:=simplify(subs(\{gb1^2=0,gb2^2=0,gal^2=0,ga2^2=0\},ln(E)));
\[ kk2 := -\gamma^2 \, D \, \delta \, (4 \, gal \, \delta^2 \, gb2 - 2 \, gal \, \delta \, gb2 \, \pi^2 \, \delta3 - 4 \, gb1 \, \pi^2 \, ga2 \, \tau1 \, \delta3 \\
+ 4 \, \pi^2 \, \delta3 \, gal \, gb2 \, \tau1 - 4 \, gb1 \, \pi^2 \, gal \, \tau1 \, \delta1 + 8 \, \delta1 \, \pi \, gal \, \delta \, ga2 + 4 \, \delta1 \, \pi^2 \, ga2 \, gb2 \, \tau1 \\
+ 8 \, \delta \, \tau2 \, \pi \, gal \, ga2 + 32 \, \pi \, gal \, \delta \, ga2 \, \tau1 - 8 \, \pi \, gal \, \delta \, ga2 \, \delta3 - 4 \, gal \, \delta^2 \, gb1 \\
- 2 \, ga2 \, \delta \, gb2 \, \pi^2 \, \delta1 + 4 \, ga2 \, \delta^2 \, gb2 + 2 \, gb1 \, \pi^2 \, ga2 \, \delta3 + 2 \, \pi^2 \, \tau^1 \, ga2 \, gb2 \]

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\[
-2\pi^2 t_1^2 \sin \theta \cos \theta + 2\sin^2 \theta \cos \theta + 2\sin \theta \cos \theta - 2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta
\]

\[
2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta - 2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta
\]

\[
2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta - 2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta
\]

\[
2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta - 2\sin \theta \cos \theta - 2\sin \theta \cos \theta + 2\sin \theta \cos \theta
\]
\[ \text{Cross4} := -\gamma^2 D \delta \, g a l \, (\delta^2 \, g b 2 \, \pi^2 - 2 \eta \, \delta \, g b l \, \pi^2 \, (2 \, \tau_1 - \delta_1 - \delta) \\
+ 2 \, \delta \, g b 2 \, \pi^2 \, (2 \, \tau_1 - \delta_1 - \delta) + 4 \, \delta^2 \, g b l - 2 \, \delta \, g b 2 \, \pi^2 \, \tau_1 + 2 \, g b l \, \pi^2 \, \tau_1 \, \delta - 4 \, \eta \, \delta^2 \, g b 2 \\
+ 4 \, \eta \, \delta^2 \, g b l - 2 \, g b l \, \pi^2 \, \delta_1^2 - 2 \, g b l \, \pi^2 \, \tau_1 \, \delta - \delta^2 \, g b l \, \pi^2 - 4 \, \delta^2 \, g b 2 \\
+ 4 \, \eta \, g b l \, \pi^2 \, \tau_1 \, (2 \, \tau_1 - \delta_1 - \delta) - 4 \, \pi^2 \, (2 \, \tau_1 - \delta_1 - \delta) \, g b 2 \, \tau_1 \\
+ 2 \, g b 2 \, (2 \, \tau_1 - \delta_1 - \delta)^2 \, \pi^2 + 2 \, \eta \, g b l \, \pi^2 \, \tau_1 \, \delta - 2 \, \delta \, g b l \, \pi^2 \, \delta_1 - 4 \, \eta \, \delta_1 \, \pi^2 \, g b 2 \, \tau_1 \\
+ 2 \, \eta \, \delta \, g b 2 \, \pi^2 \, \delta_1 - 2 \, \eta \, \delta \, g b 2 \, \pi^2 \, \tau_1 + 2 \, \eta \, g b l \, \pi^2 \, \tau_1 \, \delta + 2 \, \pi^2 \, \tau_1^2 \, g b 2 \\
- 2 \, \eta \, g b l \, \pi^2 \, (2 \, \tau_1 - \delta_1 - \delta)^2) / \pi^3 \]

Calculate magic ratio
> sol4:=solve(Cross4,eta);

\[
sol4 := \frac{\delta^2 \, \pi^2 + 2 \, \delta \, \pi^2 \, \delta_1 - 2 \, \pi^2 \, \tau_1 \, \delta - 4 \, \delta^2 + 2 \, \pi^2 \, \tau_1^2 - 4 \, \pi^2 \, \tau_1 \, \delta_1 + 2 \, \pi^2 \, \delta_1^2}{4 \, \delta^2 - \delta^2 \, \pi^2 + 2 \, \pi^2 \, \tau_1^2 + 4 \, \pi^2 \, \tau_1 \, \delta_1 - 2 \, \pi^2 \, \delta_1^2 + 2 \, \pi^2 \, \tau_1 \, \delta - 2 \, \delta \, \pi^2 \, \delta_1} \]

> simplify(sol3-sol4); 0
Define the integral used in determining $F$

\[ F := (G, t_i) \rightarrow \int_{t_i}^{t_f} G \, dt \]

Define the time intervals and the relevant value of $G$ for each integral. Also calculate the value of $F$ for each interval remembering that it contains the contribution from all of the intervals from the start of the pulse sequence.

\[ l_1 := 0; \]
\[ g_1 := 0; \]
\[ F_1 := F(g_1, l_1); \]
\[ l_2 := \delta_1; \]
\[ g_2 := -\gamma_1; \]
\[ F_2 := \text{subs}(t_f = l_2, F_1) + F(g_2, l_2); \]
\[ l_3 := \delta_1 + \delta; \]
\[ g_3 := 0; \]
\[ F_3 := \text{subs}(t_f = l_3, F_2) + F(g_3, l_3); \]
\[ l_4 := \delta_1 + \delta + \delta_2 + \delta_3; \]
\[ g_4 := \gamma_2; \]
\[ F_4 := \text{subs}(t_f = l_4, F_3) + F(g_4, l_4); \]
\[ l_5 := \delta_1 + 2 \delta + \delta_2 + \delta_3; \]
\[ g_5 := 0; \]
\[ F_5 := \text{subs}(t_f = l_5, F_4) + F(g_5, l_5); \]
\[ l_6 := 2 \delta_1 + 2 \delta + \delta_2 + \delta_3 + \delta_4 + \tau_2; \]
\[ g_6 := -\gamma_2; \]
\[ F_6 := \text{subs}(t_f = l_6, F_5) + F(g_6, l_6); \]
\[ l_7 := 2 \delta_1 + 3 \delta + \delta_2 + \delta_3 + \delta_4 + \tau_2; \]
\[ g_7 := 0; \]
\[ F_7 := \text{subs}(t_f = l_7, F_6) + F(g_7, l_7); \]
The total attenuation

\[ \ln(E) = -\gamma^2 D^2 \delta (3 \, ga1 \, \delta 1 + 8 \, ga1^2 \, \delta + 6 \, ga1^2 \, \delta 2 + 6 \, ga1^2 \, \delta 3 - 6 \, \delta \, ga1 \, ga2 + 2 \, \delta \, ga2^2 + 3 \, ga1^2 \, \delta 4 + 3 \, ga2^2 + 2 \, \delta 2 - 6 \, ga2 \, ga1 \, \delta 1 - 6 \, ga2 \, ga1 \, \delta 4 - 6 \, ga2 \, ga1 \, \tau 2 + 3 \, ga1^2 \, \delta 1 + 3 \, ga2^2 \, \delta 4 + 3 \, ga2^2 \, \tau 2) / \gamma^2 \]
\[ kk := \frac{10 \delta gg ga2}{3} + 4 \delta3 \ gab + \frac{4 \delta ga2^2}{3} + \delta1 \ g2 + \frac{8 \delta g2^2}{3} + 2 \delta2 \ g2^2 \]
\[ + 2 \delta2 \ ga2^2 + 2 \delta3 \ g2^2 + 2 \delta3 \ ga2^2 + \delta4 \ g2^2 + \tau2 \ g2^2 \]

\[ \text{collect}(%, \ g2); \]
\[ \left( \delta1 + \frac{8 \delta}{3} + 2 \delta2 + 2 \delta3 + \delta4 + \tau2 \right) g2^2 + \left( \frac{10 \ ga2 \ \delta}{3} + 4 \ ga2 \ \delta2 + 4 \ ga2 \ \delta3 \right) g2 \]
\[ + 2 \delta3 \ ga2^2 + \frac{4 \delta ga2^2}{3} + 2 \delta2 \ ga2^2 \]

\[ \text{collect}(%, \ ga2); \]
\[ \left( 2 \delta3 + \frac{4 \delta}{3} + 2 \delta2 \right) ga2^2 + \left( \frac{10 \delta}{3} + 4 \delta2 + 4 \delta3 \right) gg ga2 \]
\[ + \left( \delta1 + \frac{8 \delta}{3} + 2 \delta2 + 2 \delta3 + \delta4 + \tau2 \right) gg^2 \]
Development of binomial-like inversion sequences by the use of product operators  
G. Zheng, Allan Torres, and William S. Price

> with(linalg):
> with(plots);

Chemical shifts
> Ixz:=(abc, Omega, tau) -> abc*(Iz*cos(Omega*tau*2*Pi) + Iy*sin(Omega*tau*2*Pi));
> Izy:=(abc, Omega, tau) -> abc*(Iy*cos(Omega*tau*2*Pi) - Iz*sin(Omega*tau*2*Pi));
> Izz:=(abc, Omega, tau) -> abc*Iz;

RF pulse with phase theta and flip angle beta
> Ixz:=(abc, beta, theta) -> abc*(Iz*cos(beta) + Ix*sin(beta)*sin(theta) - Iy*sin(beta)*cos(theta));
> Ixx:=(abc, beta, theta) -> abc*(-Iz*sin(beta)*sin(theta) + Ix*(cos(beta)*sin(theta)^2 + cos(theta)^2) + Iy*sin(beta/2)^2*sin(2*theta));
> Ixy:=(abc, beta, theta) -> abc*(Iz*sin(beta)*cos(theta) + Iy*(cos(beta)*cos(theta)^2 + sin(theta)^2) + Ix*sin(beta/2)^2*sin(2*theta));

Flip angles and phases of the pulses in the binomial pulse train
> alpha3:=vector([20.8*Pi/180, 62.2*Pi/180, 131.6*Pi/180, 131.6*Pi/180, 62.2*Pi/180, 20.8*Pi/180]);
> phi3:=vector([0, 0, 0, Pi, Pi, Pi]);
> alpha5:=vector([0.087*Pi/2, 0.206*Pi/2, 0.413*Pi/2, 0.778*Pi/2, 1.491*Pi/2, 1.491*Pi/2, 0.778*Pi/2, 0.413*Pi/2, 0.206*Pi/2, 0.087*Pi/2]);
> phi5:=vector([0, 0, 0, 0, 0, Pi, Pi, Pi, Pi, Pi]);
> alpha8:=vector([a, b, c, c, b, a]);
> phi8:=vector([eta1, eta2, eta3, eta3+Pi, eta2+Pi, eta1+Pi]);

For 3-9-19
> n:=6;
> M:=Ixz(1, alpha3[1], phi3[1]);
> for i from 2 by 1 to n do
> Mx:=simplify(subs({Iy=0, Iz=0}, M)/Ix);
> My:=simplify(subs({Ix=0, Iz=0}, M)/Iy);
> Mz:=simplify(subs({Ix=0, Iy=0}, M)/Iz);
> M:=Izx(Mx, Omega, 0.0002) + Izy(My, Omega, 0.0002) + Izz(Mz, Omega, 0.0002) + Ixx(Mx, alpha3[i], phi3[i]) + Ixy(My, alpha3[i], phi3[i]) + Ixz(Mz, alpha3[i], phi3[i]);
> end do;

> Mx:=simplify(subs({Iy=0, Iz=0}, M)/Ix);
> My:=simplify(subs({Ix=0, Iz=0}, M)/Iy);
> Mz:=simplify(subs({Ix=0, Iy=0}, M)/Iz);

> p3919:=plot(Mz, Omega=-5000..5000);
For W5
> n:=10;
> M:=Ixz(1,alpha5[1],phi5[1]);
> for i from 2 by 1 to n do
  Mx:=simplify(subs({Iy=0,Iz=0},M)/Ix);
  My:=simplify(subs({Ix=0,Iz=0},M)/Iy);
  Mz:=simplify(subs({Ix=0,Iy=0},M)/Iz);
  M:=Izx(Mx,Omega,0.0002)+Izy(My,Omega,0.0002)+Izz(Mz,Omega,0.0002);
  Mx:=simplify(subs({Iy=0,Iz=0},M)/Ix);
  My:=simplify(subs({Ix=0,Iz=0},M)/Iy);
  Mz:=simplify(subs({Ix=0,Iy=0},M)/Iz);
  M:=Ixx(Mx,alpha5[i],phi5[i])+Ixy(My,alpha5[i],phi5[i])+Ixz(Mz,
  alpha5[i],phi5[i]);
end do;
>
For a-b-c-c-b-a ph1-ph2-ph3-(ph3+Pi)-(ph2+Pi)-(ph1+Pi)
> n:=6;
> M:=Ixz(1,alpha8[1],phi8[1]);
> for i from 2 by 1 to n do
  Mx:=simplify(subs({Iy=0,Iz=0},M)/Ix);
  My:=simplify(subs({Ix=0,Iz=0},M)/Iy);
  Mz:=simplify(subs({Ix=0,Iy=0},M)/Iz);
  M:=Izx(Mx,Omega,0.0002)+Izy(My,Omega,0.0002)+Izz(Mz,Omega,0.0002);
  Mx:=simplify(subs({Iy=0,Iz=0},M)/Ix);
  My:=simplify(subs({Ix=0,Iz=0},M)/Iy);
  Mz:=simplify(subs({Ix=0,Iy=0},M)/Iz);
  M:=Ixx(Mx,alpha8[i],phi8[i])+Ixy(My,alpha8[i],phi8[i])+Ixz(Mz,
  alpha8[i],phi8[i]);
end do;
>
Optimize the pulse durations and phases
> with (Optimization);
> print([LABEL REFERENCE NOT SUPPORTED]);
> data := [[50, 0], [1500, -1],[2000,-1],[3000,-1],[3500,-1]];
> Mzoff:=Omega->Mz;
> residues := map((d)->(eval(Mzoff(Omega), Omega = d[1]))-d[2], data);
> sol := LSSolve(residues);
Reference

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List of Publications

Parts of the work presented in this Thesis have appeared in the following publications:


