Steady state effects in PGSE NMR diffusion experiments

Tim Stait-Gardner, P.G. Anil Kumar, William S. Price *  
Nanoscale Organisation and Dynamics Group, College of Health and Science, University of Western Sydney, Penrith South DC NSW 1797, Australia

1. Introduction

The pulsed gradient spin-echo (PGSE) NMR technique is now the method of choice for measuring self-diffusion (aka translational diffusion) and its applications have grown exponentially in recent years ranging from probing surfactants and organometallic systems through to MRI measurements in conjunction with clinical medical applications [1–4]. The importance and dominance of this technique lies in it being able to provide the diffusion coefficient \(D\) of multiple species in a sample simultaneously and in a non-invasive manner. The simplest commonly used Hahn echo based PGSE sequence is shown in Fig. 1 [5].

Briefly, the gradient pulses, \(g\), are collinear with the main magnetic field \(B_0\) and result in a spatial variation in magnetisation, \(B_1 = B_0 + g z\). The first magnetic gradient pulse of magnitude \(g\) and duration \(\delta\) spatially encodes the coherent transverse magnetisation into a helix with a pitch proportional to the inverse of the 'area' of the gradient pulse i.e., \(q = 2\pi/|\gamma g|\) m. Diffusion of the spins attenuates the helix diameter before an identical gradient pulse of effectively negative amplitude unencodes the helix. Greater diffusion or larger gradient pulses result in a smaller acquired signal \((S)\). Normalising out the effects of spin–spin relaxation \((T_2)\), leads to the signal attenuation for a single freely diffusing species being described by [5,6]

\[
E = \frac{5}{5-\delta^2} \exp \left( -\frac{\gamma g^2 D \delta^2}{2} (A - \frac{\delta^2}{3}) \right).
\]  

\(D\) can be obtained by running the PGSE sequence with a number of different gradient strengths and using a non-linear least squares fit of Eq. (1) to the resulting data.

Being a quantitative measurement, it is important that each application of the PGSE sequence starts from a reproducible state. Experimentally this normally equates to waiting for thermal equilibrium before the initial \(\pi/2\) rf excitation pulse is applied. Consequently, the recycle delay \(T_R = T_{D0} + Acq\), where \(T_{D0}\) is the prescan delay, see Fig. 1) is normally set to be at least five times the spin-lattice relaxation time i.e., \(T_R \geq 5 \times T_1\) and thus diffusion measurements typically take on the order of tens of minutes. Such a delay is often not adhered to with some published measurements being performed with shorter recovery delays (e.g., \(3 \times T_1\)).

The range of application of diffusion measurements would be considerably expanded (e.g., towards protein folding kinetics) if the measurement time could be reduced. Many approaches to fast diffusion measurements have been suggested and can be loosely grouped into singleshot and oneshot sequences. A singleshot sequence is a complete diffusion measurement carried out in a single scan and is based on a multi-echo approach whereas a oneshot sequence has a reduced phase cycling such that ideally one q-space point is applied in a single scan. These techniques often require good signal-to-noise (SN) and generally have other drawbacks such as loss of chemical shift information or a limited suitability for studying diffusion in restricted systems because of poorly defined diffusion time.

Packer [7] proposed a modification to the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence in which \(\pi\) pulses are inserted at each echo maximum and in theory it is possible to determine an estimate for the diffusion coefficient by monitoring the echo amplitudes in a single shot. Song et al. [8] reported a method related to that of Packer based on a modified CPMG sequence. Van Gelderen and co-workers [9] proposed a spin-echo based experiment in which a series of gradient echoes are created, however the ability of this method to retain chemical shift information is limited. Doran and Decorps [10] used a sequence of small angle excitations in the presence of a read gradient which is then

* Corresponding author. Fax: +61 2 4620 3025.
E-mail address: w.price@uws.edu.au (W.S. Price).

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commonly used in NMR, for example in T₁ measurements, see Ref. [25] and references therein) before the first rf pulse (normally π/2, but we also consider the case of an arbitrary nutation angle θ). It might be reasoned that the S/N per unit time would be optimised by choosing θ between π/2 and π when T₀ < 5 × T₁ since although the transverse magnetisation component is reduced by cos θ, the longitudinal component will be sin θ (which is negative due to π/2 < θ < π instead of zero in the case of a π/2 pulse) which is inverted (becomes positive) after the π pulse. Thus there is an optimum flip angle (‘Ernst angle’) between π/2 and π. By far the greatest contribution to S/N, in cases where one pulses faster than 5 × T₁ is from this faster pulsing. Thus, we recommend sticking with a π/2 pulse since the optimum angle requires an accurate value of T₁. The following analysis is carried out for a θ pulse, however, to show that whether chosen deliberately or simply as a result of imperfect rf homogeneity a steady state will still be reached.

In examining whether a steady state is reached the evolution of both the transverse and longitudinal components of the magnetisation have to be traced through the sequence. The analysis will show the following. First it is shown, starting with an arbitrary initial magnetisation aligned along the z axis, that there may be some residual transverse magnetisation left at the beginning of the next transient. It is then shown that this residual transverse magnetisation is destroyed by diffusive attenuation before acquisition and has no effect of the longitudinal component of the magnetisation at the beginning of the next transient. It is then sufficient to analyse whether the longitudinal component reaches a steady state after a sufficient number of transients and it is shown that indeed it does. In brief, the residual transverse magnetisation is wound into a fine (highly spatially periodic) helix. The π/2 pulse transforms this into a sinusoidally varying transverse magnetisation and a sinusoidally varying longitudinal magnetisation. The longitudinal component is unaffected by the gradient and is only inverted by the π pulse and will attenuate via diffusion before the next pulse iteration. The transverse component can be viewed as the sum of two magnetisation helices of opposite chirality. The transverse magnetisation helices are wound finer or coarser depending upon their chirality but spend enough time with a pitch fine enough to cause their complete attenuation by the time of acquisition. These effects are explained in detail below.

After the initial θ pulse the (purely) longitudinal magnetisation M will transform into longitudinal (i.e., Mₗ = M cos θ) and transverse (Mₘₗ = M sin θ components. This transverse component will be encoded and decoded by the diffusion encoding gradients, resulting in its diffusive attenuation and at the beginning of acquisition its magnitude will be

\[
Mₘₗ = M \sin \theta \exp \left( -\gamma^2 g^2 D \delta^2 \left( \Delta - \frac{\delta}{3} \right) \right) \exp \left( \frac{-2A}{T₂} \right).
\]

After acquisition and before the crusher gradient pulse it will have further attenuated.

We now trace the magnetisation through the second application of the pulse sequence. The crusher gradient pulse encodes any residual transverse magnetisation into a helix with a pitch at least as tight as the helix encoded by the maximum strength gradient pulse used in the PGSE measurement: \( \delta g < \delta g_{\text{max}} \) (where \( \delta g \) is the duration and \( g \) the amplitude of the crusher gradient pulse). This helix has a pitch fine enough to cause complete attenuation through diffusion during the period \( A \). Immediately prior to the application of the θ pulse the longitudinal magnetisation is denoted by \( M' \) and there is now transverse magnetisation (albeit encoded in a helix) proportional to \( \Phi/2 \Phi_{\text{max}} \). After the θ pulse the transverse magnetisation has contributed a term proportional to \( \sin \theta \cos(2\pi g \delta t) \) to the longitudinal magnetisation and the
transverse magnetisation is now proportional to \( \cos \theta \cos(z_0g \gamma_{\text{c}}) + i \sin(z_0g \gamma_{\text{c}}) \).

The longitudinal component is unaffected by gradient pulses and has a period of \( \lambda \) to attenuate via diffusion before the \( \pi \) pulse; this is enough to cause it to attenuate completely. Now consider the transverse components. Looking just at the \( \cos \theta \cos(z_0g \gamma_{\text{c}}) \) term we see that it is composed of the sum of the two helices

\[
\cos \theta \cos(z_0g \gamma_{\text{c}}) = \frac{1}{2} \cos \theta \{ \cos(z_0g \gamma_{\text{c}}) + i \sin(z_0g \gamma_{\text{c}}) \} + \frac{1}{2} \cos \theta \{ \cos(-z_0g \gamma_{\text{c}}) + i \sin(-z_0g \gamma_{\text{c}}) \}.
\]

During the \( \pi - \pi \) period (where \( \tau \gg \lambda \)) one helix will be wound into an even finer helix causing it to attenuate faster. The other will be wound into a coarse helix causing it to attenuate slower. The coarser helix will still spend a period \( \lambda \) with pitch \( 2\pi \gamma_{\text{c}} \gamma_{\text{c}} \) resulting in it also completely attenuating during this time. The same happens with the \( i \sin(z_0g \gamma_{\text{c}}) \) term. Thus by the time of acquisition there is no magnetisation remaining from the initial transverse helix. Hence only the initial longitudinal component needs to be taken into account in determining whether a steady state is reached.

Suppose that the longitudinal magnetisation is \( M \) before the \( \theta \) pulse. For greater generality we will now replace the \( \pi/2 \) pulse with a pulse of arbitrary angle \( \theta \). The longitudinal magnetisation after the \( \theta \) pulse is \( M \cos \theta \). The Bloch equations \[26\] result in the longitudinal magnetisation evolving according to

\[
M_l(t) = M_0 + (M - M_0) \exp(-t/T_1)
\]

where \( M_0 \) is the equilibrium (longitudinal) magnetisation. The longitudinal magnetisation at acquisition (evolving as \( M \cos \theta \) through \( \tau \), a \( \pi \) pulse and another \( \tau \)) is

\[
M_l(2\tau + T_\theta) = M_0 - 2M_0 + (M \cos \theta - M_0) \exp(-\tau/T_1) \exp(-\tau/T_1).
\]

This is the magnitude of the signal at acquisition and is dependent on \( M \). The longitudinal magnetisation just before the next application of the \( \theta \) pulse is obtained by allowing the magnetisation in Eq. \((5)\) to evolve for a time \( T_{\text{Acqu}} + T_\theta \) giving

\[
M_l(2\tau + T_\theta + T_\theta) = M_0 - 2M_0 + (M \cos \theta - M_0) \exp(-\tau/T_1) \exp(-\tau/T_1) \exp(-\tau/T_1).
\]

This is the initial longitudinal magnetisation used in the second transient. There is no transverse magnetisation except perhaps a tight helix of residual magnetisation wound up by the crusher gradient which will diffuse away during the next transient.

Defining

\[
a = M_0 - M_0(2 - \exp(-\tau/T_1)) \exp(-\tau/T_1)
\]

and

\[
b = - \cos \theta \exp\left(-\frac{2\tau + T_\theta}{T_1}\right).
\]

Eq. \((6)\) can be expressed as

\[
M_2 = M_l(2\tau + T_\theta) = a + bM_0.
\]

Notice that both \( a \) and \( b \) are independent of \( M \). The magnetisation, \( M_3 \), just before the \( \theta \) pulse of the third transient is

\[
M_3 = a + bM_2 = a + b(a + bM_0) = a + ab + b^2M_0.
\]

The magnetisation, \( M_n \), just before the \( nth \) \( \theta \) pulse is

\[
M_n = a \left( \frac{1 - b^{n-1}}{1 - b} \right) + b^{n-1}M_0.
\]

To see Eq. \((11)\) is true note that it holds for \( n = 1 \), in which case \( M_1 = M \). Assuming that \( M_n \) holds for \( n, M_{n+1} \) is given as

\[
M_{n+1} = a + bM_n = a + b\left( \frac{1 - b^{n-1}}{1 - b} \right) + b^{n-1}M
\]

\[
= a \left( \frac{1 - b^{(n+1)-1}}{1 - b} \right) + b^{(n+1)-1}M,
\]

and thus Eq. \((11)\) is seen to hold true via induction. Since \( |b| < 1 \) (see Eq. \((8)\)), it follows that after a sufficient number of dummy scans \( b^{n+1} = 0 \) (because \( \lim_{n \to \infty} b^n = 0 \)) and a steady state longitudinal magnetisation, denoted \( M_{\infty} \), is reached

\[
M_{\infty} = a \left( \frac{1 - b^{n+1}}{1 - b} \right) + b^{(n+1)-1}M
\]

Notice that Eq. \((13)\) is independent of the initial magnetisation, \( M \).

Thus, a steady state will be reached irrespective of the \( T_\theta \) chosen but the value of \( T_\theta \) will affect the number of dummy scans required to reach the steady state. Thus, given sufficient dummy scans, it is unnecessary to wait \( 5 \times T_\theta \). For \( T_\theta \), Eqs. \((8)\) and \((11)\) show that when \( \theta = \pi/2 \) the steady state is reached after just one transient. However, the \( \pi/2 \) pulse is unlikely to be perfect and there is likely to be some degree of field inhomogeneity in the sample. Thus it is best to use more than one dummy scan – in this work 16 scans were used.

Assuming a steady state, the optimum pulse rate in situations of low \( S/N \) can be calculated. The signal is proportional to the transverse magnetisation after the \( \theta \) pulse given by

\[
S_{\text{Acqu}}(\theta, T) = M_0 \sin \theta \left( \frac{1 - \exp(-T/T_1)}{1 + \cos \theta \exp(-T/T_1)} \right)
\]

where \( T \) is the time from the initial rf excitation pulse to acquisition. This follows from Eq. \((13)\) (the \( \sin \theta \) term results from the signal being proportional to the magnitude of the transverse magnetisation after the \( \theta \) pulse). If the sequence is run \( n \) times and summed the magnitude of the signal will \( n \) times larger than running the sequence just once. The noise increases as the square root of the number of scans \( \sqrt{n} \). Thus the \( S/N \) improves as the square root of the number of runs. If the sequence is run faster, fitting \( m \) scans where previously there were \( n \) (where \( m > n \)) into the time period \( nT \) (although keeping the acquisition times the same), and thus reducing the time of a single scan from \( T \) to \( \beta T \) (where \( \beta = m/n \)), the \( S/N \) is proportional to

\[
\sqrt{m} S_{\text{Acqu}}(\theta, \beta T).
\]

The increase in efficiency, \( Q \), gained by running the sequence \( m \) times during time \( nT \) with flip angle \( \theta \) than running it \( n \) times with a \( \pi/2 \) flip angle is given by

\[
Q = \frac{\sqrt{m} S_{\text{Acqu}}(\theta, \beta T)}{\sqrt{n} S_{\text{Acqu}}(\pi/2, T)}.
\]

If the value of \( Q \) for some value of \( \theta \) and \( \beta \) is greater than one then using these parameters is an improvement over pulsing at \( \pi/2 \) and waiting \( 5 \times T_\theta \). \( Q \) is given by

\[
Q = \beta^{-1/2} \sin \theta \left[ 1 + (1 - 2 \exp(\pi/2T_1)) \exp(-\beta \pi T_1/2) \right] \left[ 1 + (1 - 2 \exp(\pi/2T_1)) \exp(-T/T_1) \right]^{-1/2}.
\]

We want to find the optimum values of \( \beta \) and \( \theta \) such that Eq. \((17)\) is maximised. These can be found by solving

\[
\frac{dQ}{d\theta} = 0
\]
and
\[ \frac{dQ}{d\beta} = 0. \]  

Eq. (18) gives the optimum pulse angle to be
\[ \cos \theta = -\exp \left( \frac{\tau + 5T_1}{T_1} \right) \]  

where \( T = \tau + 5T_1 \). Eq. (19) gives a less useful expression. The optimum \( \beta \) can be found from Fig. 2.

3. Experimental

Measurements were carried out on a Bruker Avance 500 wide-bore spectrometer equipped with a BBI probe. NMR samples lengths of less than 1 cm were employed by using 5 mm Shigemi tubes (Shigemi, Japan) to ensure high rf homogeneity. The temperature was 25°C for all experiments.

The parameters used during the experiments were as follows: \( A \) was set to 100 ms for the experiments on residual HDO in D_2O and to 160 ms for the experiments on 10% isopropanol and 10% methanol in D_2O which from now on will be referred to as the alcohol sample. \( \delta \) was set to 1 ms for the experiments done solely on residual HDO in D_2O and 1.5 ms for the experiments performed on the alcohol sample. The gradients were ramped from 0.027 G/cm to 0.43 G/cm in increments of 0.027 G/cm for the experiments on residual HDO in D_2O and for a total of 16 measurements. For the alcohol sample the gradients were ramped from 0.013 G/cm to 0.42 G/cm in increments of 0.013 G/cm for a total of 32 measurements. Rectangular pulses were used in all cases. Only one transient was acquired for each separate gradient strength. Sixteen dummy scans prior to the first scan were used to ensure a steady state was reached. The dummy scans occur only at the beginning of the experiment before the diffusion encoding gradients are incremented. No dummy scans occur between gradient increments. The crusher gradient had a magnitude of 0.43 G/cm and duration of 4 ms for residual HDO in D_2O and 3 ms for the alcohol sample. Sufficient time was given after each gradient pulse to ensure eddy currents had decayed away before application of any rf pulses. An acquisition time (Acq) of 0.63 s was used in all cases. The diffusion coefficient was then obtained by fitting Eq. (1) to the data using (OriginLab, MA) Levenberg-Marquardt non-linear curve fitting.

4. Results and discussion

The \( T_1 \) of residual HDO in D_2O was measured using the inversion recovery sequence and was found to be 12.7 s. Twelve diffusion experiments were then carried out on this sample, each with a different recycle time, \( T_D \). The prescan delay (\( T_D \)) were, in units of \( T_1 \): 5, 4, 3, 2, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128. The diffusion coefficient obtained from each experiment was then plot-
A simple experimental system unless intermolecular multiple quantum coherences could be generated in such a system. It is however, difficult to see how multiple quantum coherences could be generated in such a simple experimental system unless intermolecular multiple quantum coherences were involved [28,29].

For the alcohol sample twice as many data points (32) were taken. The results of the experiment are presented in Fig. 5A. Notice that isopropanol and residual HDO seem relatively well behaved while methanol deviates near $T_1$. We appear to get better results for water in the alcohol sample than when it is by itself and speculate that this is simply caused by the parameters $\Delta$ and $\delta$ being larger which would cause the water peak to attenuate faster. Any multiple quantum coherences will attenuate even faster still. Thus these larger values of $\Delta$ and $\delta$ would reduce the effects of intermolecular dipolar couplings, if indeed these couplings are present.

Notice the hump in the variation of the diffusion of methanol with respect to $T_0$. Interestingly the methanol hump, when plotted in units of $T_1$ as above, occurs in a similar region to the HDO hump when plotted in units of $T_1$ despite their different $T_0$s; for residual HDO $T_1 = 9.7$ s; for methanol, 7.3 s; for the CH peaks of isopropanol, 6.9 s; for the CH$_3$ peaks of isopropanol, 2.9 s.

The relative diffusion coefficient (the measured diffusion coefficient divided by the diffusion coefficient obtained at long $T_D$) is plotted versus $T_D/T_1$ in Fig. 5B. The deviation of the methanol diffusion coefficient becomes even more apparent in this plot. However, if the first 12 data points in each diffusion experiment are omitted and then the diffusion coefficient is calculated by fitting the remaining data to a double exponential (see Eq. (21)) then the resulting variation in measured diffusion coefficient versus $T_D/T_1$ is small (Fig. 6A).

Plotting Fig. 6A with relative diffusion coefficients instead of absolute values on the same scale as Fig. 5B gives Fig. 6B. From this graph it can be seen that the measured diffusion coefficients remain within 2% of the actual values regardless of $T_D$.
The SS-PGSE sequence can significantly speed up diffusion measurements but care must be exercised in its application. It is best to ramp the gradients from an intermediate to a high value as opposed to from low to high as this reduces the anomalous change in the diffusion coefficient with respect to faster recycle times. Choosing larger values of $D$ and $d$ may also be advisable.

5. Conclusions

It is clear from the experimental results that the sequence does not behave as simply as the theory suggests. There is a rule-of-thumb that one wait $5 \times T_1$ for the sample to relax back to equilibrium before the next scan. This rule is often ignored and recycle delays of $3 \times T_1$ are commonly encountered in the literature. The discrepancy between the theory and experiment above should act as a warning that care should be taken when deliberately deciding to pulse faster. Despite the unexpected variation in measured diffusion coefficient at faster recycle times the SS-PGSE sequence, at least on the samples tested, can be used to obtain diffusion coefficients quickly and relatively accurately if the gradients are ramped from intermediate to high values. It is quite obvious when the results deviate as a single exponential does not fit well to the data. The unexpected and interesting observed behaviour of the SS-PGSE sequence will be investigated in a future paper.

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References