Effects of off-resonance spins on the performance of the modulated gradient spin echo sequence

Igor Serša *, Franci Bajd, Aleš Mohorič

Jožef Stefan Institute, Jamova 39, Ljubljana 1000, Slovenia
Faculty of Mathematics and Physics, University of Ljubljana, Jadranska 19, Ljubljana 1000, Slovenia

ABSTRACT

Translational molecular dynamics in various materials can also be studied by diffusion spectra. These can be measured by a constant gradient variant of the modulated gradient spin echo (MGSE) sequence which is composed of a CPMG RF pulse train superimposed to a constant magnetic field gradient. The application of the RF train makes the effective gradient oscillating thus enabling measurements of diffusion spectra in a wide range of frequencies. However, seemingly straightforward implementation of the MGSE sequence proved to be complicated and can give overestimated results for diffusion if not interpreted correctly. In this study, unrestricted diffusion in water and other characteristic materials was analyzed by the MGSE sequence in the frequency range 50–3000 Hz using a 6 T/m diffusion probe. First, it was shown that the MGSE echo train acquired from the entire sample decays faster than the train acquired only from a narrow band at zero frequency of the sample. Then, it was shown that the decay rate is dependent on the band’s off-resonance characterized by the ratio Δω₀/ω₁, and that with higher off-resonances the decay is faster. The faster decay therefore corresponds to a higher diffusion coefficient if the diffusion is calculated using standard Stejskal-Tanner formula. The result can be explained by complex coherence pathways contributing to the MGSE echo signals when |Δω₀|/ω₁ > 0. In a magnetic field gradient, all the pathways are more diffusion attenuated than the direct coherence pathway and therefore decay faster, which leads to an overestimation of the diffusion coefficient. A solution to this problem was found in an efficient off-resonance signal reduction by using only zero frequency filtered MGSE echo train signals.

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1. Introduction

Measurements of translational molecular dynamics gives a valuable insight in the structure of various materials ranging from polymers and porous materials to complex biological systems [1–3]. There are different approaches how to measure the dynamics [4]. The most common one is based on the use of the pulsed gradient spin echo (PGSE) sequence, which relies on the use of two magnetic field gradient pulses positioned symmetrically to the RF refocusing pulse [5]. In the approach the PGSE sequence is repeated with increasing amplitudes of the gradient pulses G, while the pulse duration δ and the diffusion-encoding delay Δ remain constant. By the approach, a series of differently attenuated echo signals is measured, which then via Fourier transform of the signals enable calculation of a probability function for an average diffusion spin displacement \( \hat{R} \) in time Δ, also known as an average propagator \( P_R(\hat{R}, \Delta) \) [6–8]. Other possible processing options of the PGSE data include the Stejskal-Tanner plot (\( \ln(S_{C211}) \) vs. \( G^2 \)) or inverse Laplace transformation. The propagator approach, which can be considered as displacement spectroscopy, is characterized by the diffusion-encoding delay Δ. In principle, it gives very different results with different delays Δ if there is a restriction of diffusive motion, as for example in confined geometries. Therefore, it is desirable to probe morphology of materials also with different delays Δ. For that, another approach of probing translational dynamics, namely diffusion spectroscopy, proved to be more efficient [9–12].

Diffusion spectroscopy relies on application of modulated gradients (MG) \( G(t) \) that cause spin dephasing \( F(t) = \int_0^\gamma G(t')dt' \) [9]. The cumulant expansion in Gaussian approximation associates the dephasing with a diffusion signal attenuation via the relation \( \ln(S_{C211}) = -\pi^{-1} \int_0^\gamma D(\omega)F(\omega)^2d\omega \) where \( D(\omega) \) is the modulation-frequency-dependent diffusion constant equal to a
Fourier transform of the velocity autocorrelation function and $F(\omega)$ is the spin dephasing spectrum [10]. To extract $D(\omega)$ at a particular frequency $\omega$, one needs to shape modulated gradients in such a way that $F(\omega)$ becomes a function with only one narrow peak at the frequency $\omega$; ideally $F(\omega)$ becomes Dirac's delta function. Latter can unfortunately not be achieved, however, a reasonably good approximation of it is obtained with the use of oscillating gradients (OG). Here the frequency of gradient oscillations $G(t) = G_0 \cos(\omega t)$ also corresponds to the modulation frequency at which the diffusion constant is measured. The diffusion spectrum can then be measured simply by changing oscillation frequency of the gradients over a range of frequencies. To make the measurements of signal attenuation more robust and insensitive to $T_2$ relaxation, oscillating gradients are often combined with the use of a spin echo thus obtaining an oscillating gradient echo train (OGSE) sequence [11,13,14]. The OGSE sequence has some advantages, such as low SAR, that enables its use also in clinical trials, and it is insensitive to off-resonance spins [15,16]. However, OGSE poses very high demands on gradient hardware, especially when measuring high-frequency part of a diffusion spectrum. For that, gradients need to switch very rapidly, and then diffusion attenuation decreases. Therefore, with high frequencies the gradient amplitude must be increased in order to compensate the attenuation decrease. Because of the limitations it is difficult to run OGSE experiments with frequencies above 1 kHz.

The modulation frequency limitations with OGSE can be overcome by using a modulated gradient spin echo (MGSE) sequence [12,17]. The advantage of the MGSE sequence over the OGSE sequence stems from the use of a constant actual gradient, while the corresponding effective gradient is oscillating. This is achieved by superimposing the CPMG RF pulse train to a constant magnetic field gradient. Each refocusing RF pulse in the train changes the sign of the effective gradient thus making the effective gradient oscillating with a period equal to double the inter-echo time, i.e., double the spacing between refocusing RF pulses. The gradient modulation is in the MGSE sequence obtained by the use of the CPMG RF pulse train and not by rapid switching of high gradients, therefore, higher modulation frequencies can be reached. These are limited by the highest attainable gradient that in combination with relaxation and diffusion properties of the sample produces reasonable MGSE echo train attenuation enabling reliable measurement of the diffusion spectrum.

Recently Álvarez et al. proposed an interesting alternative to the MGSE sequence, namely the selective-dynamical-recoupling (SDR) sequence [18]. MGSE and SDR sequences have in common use of multiple spin echoes in presence of a constant magnetic field gradient, constant time to the last echo, and variable modulation frequency of the effective gradient. However, the SDR sequence uses the CPMG pulse train only in the first part of the sequence and Hahn echo in the second. Its modulation function has therefore more frequency components so that the sequence, in contrast to the MGSE sequence, is not very convenient for a diffusion spectrum measurement. Its design is better suited for a measurement of a correlation time and consequently for a pore size determination that is enabled by differences between Hahn and CPMG echo attenuation for a diffusing particle in a confined geometry. The SDR sequence can also be performed by relatively low gradients and with fewer RF pulses than the MGSE sequence. Interpretation of the SDR sequence results is based on a model assuming the spectral density of a Lorentzian shape, while diffusion spectra measured by the MGSE sequence enable further analysis by various models.

If the previously mentioned highest attainable gradient amplitude would be the only MGSE sequence constraint, then application of the MGSE sequence would be fairly easy and efficient. However, our recent results on diffusion spectra of various liquids proved that there are still other important MGSE parameters with an unclarified role. Namely, all the measured spectra had an unexpected increase with frequency, which also depended on the sample size and on the way how diffusion attenuation was attained; i.e., high gradients with short echo train duration vs. low gradients with long echo train duration. Furthermore, flatter spectra were obtained with smaller samples and low gradients with low echo train durations. This indicates that the origin of the unexpected diffusion spectra could be in off-resonance spins or more precisely in a ratio of their off-resonance and power of RF pulses $\Delta\omega_0/\omega_0$.

With early implementations of the MGSE sequence, also due to lack of accuracy, it was more difficult to detect the off-resonance artefacts in diffusion spectra. In some studies, for example in a MGSE study of granular materials [19] the applied gradients were very low, so that off-resonance effects were negligible. In others [12], where porous materials were studied, sequence artifacts were masked by a significant frequency dependency of the diffusion spectra. That the results of the MGSE sequence are much different than expected became clear when MGSE was used to study diffusion in bulk liquids [20]. These results exhibited a moderate diffusion increase with an increase of MGSE frequency, which was rather unexpected. The MGSE method in our earlier studies was using only amplitude of the last echo to determine a diffusion spectrum. In addition, the method was performed with only up to four different values of the constant gradient depending on the frequency range so that there was a difficulty of correct interpretation of measurements from different frequency ranges. Due to these limitations the method was more prone to systematic errors than our later modifications of the MGSE method, which along with our unawareness of off-resonance effects contributed to invalid conclusions in a study presented in [20], where the off-resonance sequence artefacts were interpreted as “slow chain-like dynamics” in bulk water.

In this study, the presence of off-resonance artefacts in the MGSE sequence is confirmed by analyzing results of the MGSE sequence with various off-resonances $\Delta\omega_0/\omega_0$ on a water sample. In the end, a method for a significant reduction of off-resonance signals in the MGSE sequence is presented and also tested on samples with different diffusion properties. The test results confirmed that with the available hardware the improved MGSE sequence can produce reliable results with MGSE frequencies up to 3 kHz. Effects of off-resonance spins and reduction of their contribution to the MGSE signals were already analyzed and discussed in [21], however, the present study provides more thorough analysis of the effects.

2. Materials and methods

2.1. MGSE sequence with on-resonance spins

According to [12], the MGSE pulse is composed of a sequence of RF pulses combined with magnetic field gradient pulses or waveforms that periodically modulate the spin phase in order to get the spin echo attenuation proportional to the power spectrum of molecular displacement (DPS) or the velocity autocorrelation spectrum, depending on the applied sequence. In this study, a constant gradient variant of the MGSE sequence was used [21], where a standard CPMG RF pulse train is run simultaneously with a constant magnetic field gradient $G_0$ (Fig. 1a). Let us assume RF pulses are of very high amplitudes in comparison to off-resonances of spins in the sample $(\Delta\omega_0 << \omega_0)$. In addition, diffusion is assumed unrestricted and obeys Fick's law. Such a diffusion model describes molecular translational motion with a flat diffusion spectrum. Under these assumptions, according to Carr and Purcell’s formula for diffusion-induced spin-echo attenuation [22], the signal amplitude in consecutive echoes of the MGSE sequence is equal to

$$F(\omega) = \frac{\omega^2}{\omega_0^2} \sin^2 \left(\frac{\omega t}{2}\right)$$

where $\omega$ is the frequency of oscillating gradients, $G(t) = G_0 \cos(\omega t)$, and $\omega_0$ is the Larmor frequency of the system. In this case, the diffusion constant can be calculated as

$$D = \frac{1}{2\pi^2} \frac{\omega_0^2}{\omega^2}$$

This expression is valid for low diffusion rates and can be used to estimate the diffusion constant in a sample.
Here $i$ is the echo number, $N$ is the total number of spin-echoes in the MGSE sequence and $D$ is the diffusion spectrum at a frequency of $1/(2\tau)$, where $\tau$ is the inter-echo time. The second term of the exponent in Eq. (1) includes the effect of $T_2$ relaxation during the echo time $i\tau$. In case of the repeated MGSE experiment with magnetic field gradient set to zero (standard CPMG experiment) the diffusion term in the exponent is zero yielding:

$$S_0(G_N, i\tau) = S_0(0) \exp \left( -\frac{2G_N^2 \tau^2 D}{12} \frac{i\tau}{T_2} \right); \quad i = 1 \ldots N.$$  

(1)

The effect of the relaxation on the echo signals can then be removed by dividing the MGSE echo amplitude by the CPMG echo amplitude:

$$y_i = \frac{S_i(G_N, i\tau)}{S_0(0)} = A \exp(-i\Delta b_n D); \quad i = 1 \ldots N,$$

(3)

where

$$A = \frac{S_0(G_N)}{S_0(0)} \quad \text{and} \quad \Delta b_n = \frac{G_N^2 \tau^3}{12}.$$  

(4)

Each refocusing RF pulse in the MGSE sequence changes the magnetization phase sign. The effect of the refocusing RF pulse is therefore equivalent to a sign change of the applied gradient so
that the effective gradient in MGSE oscillates with a period equal to twice the inter-echo time (Fig. 1a). Such MGSE experiment therefore corresponds to a diffusion measurement at a frequency of

$$v = \frac{1}{2\pi} = \frac{N}{2T_{\text{MGSE}}}$$

where $T_{\text{MGSE}}$ is the duration of the MGSE echo train. The diffusion spectrum at the MGSE frequency $v$ can be calculated by fitting the model function given by Eq. (3) to the experimentally obtained relaxation-compensated echo amplitudes $y_i = \tilde{S}(\tilde{G}_N, it)/\tilde{S}(0, it)$ (symbol ~ denotes experimental values). This can be done either by a commercial program for model function fitting to the experimental data or by solving the overdetermined system of equations $y_i = A \exp(-i\Delta b_{ny}) (N$ equations, two parameters $A$ and $D$). According to [23] latter gives a solution equal to

$$a = \sum_{i=1}^{N} \frac{1}{\sigma_i^2}, \quad b = \sum_{i=1}^{N} i\Delta b_{ny_i}/\sigma_i^2, \quad c = \sum_{i=1}^{N} i^2\Delta b_{ny_i}^2/\sigma_i^4, \quad d = \sum_{i=1}^{N} -\ln(y_i)/\sigma_i^2,$$

$$e = \sum_{i=1}^{N} i\Delta b_{ln}(y_i)/\sigma_i^2, \quad A = \exp\left(\frac{b - cd}{ac - b^2}\right), \quad \sigma_A = \sqrt{\frac{c}{ac - b^2}}, \quad D = \frac{ae - bd}{ac - b^2}, \quad \sigma_D = D = \sqrt{\frac{a}{ac - b^2}}$$

Here $\sigma_A$ and $\sigma_D$ are fits error of $A$ and $D$, respectively, and $\sigma_i$ is the RMS noise of $\ln(y_i)$. The latter can be estimated by $\sigma_i = \sqrt{(\sigma_C^2/\tilde{S}(\tilde{G}_N, it)^2) + (\sigma_C/\tilde{S}(0, it)^2)}$ where $\sigma_C$ is signal noise in $\tilde{S}(\tilde{G}_N, it)$ and $\tilde{S}(0, it)$. To obtain reliable fits for the determination of $D(v)$, it is desirable that attenuation of the MGSE echo train is not too high (signal at the end of the train is almost identical to the initial signal) or too high (signal at the end of the train is of the noise level). In two-point diffusion experiments, determination of $D$ is most accurate when $b = 1.29/D$ [24]. In addition, it is desirable to keep the attenuation constant with all measured frequencies. With a constant gradient this is not possible as the attenuation decreases with increasing frequencies. However, this can be done if the gradient amplitude is for each MGSE frequency adjusted according to

$$G_N = N\Delta G; \quad \Delta G = \sqrt{-\frac{12\ln(f)}{\sqrt{12\pi^2T_{\text{MGSE}}}}},$$

In Eq. (7) $f$ is a parameter controlling the MGSE echo train attenuation and corresponds to the ratio between the amplitudes of the last and first echo of the MGSE echo train, while $D_p$ is the predicted diffusion spectrum average value of the measured sample. Using the gradient adjustment in Eq. (7), the parameter controlling diffusion attenuation changes to

$$\Delta b_{ny} = \frac{\Delta b}{N}; \quad \Delta b = \frac{\Delta G^2T_{\text{MGSE}}^2}{12}.$$

In a MGSE experiment, a diffusion spectrum is measured by running a set of MGSE sequences in a broader range of frequencies. In order to keep the influence of relaxation effects on the measured diffusion spectrum constant over the entire frequency range, $T_{\text{MGSE}}$ is kept constant in all the sequences, while $N$ is increasing in the sequences according to Eq. (5) to measure diffusion in the entire frequency range.

2.2. MGSE sequence with off-resonance spins

In a MGSE experiment, spins are on average off-resonance ($\Delta \omega_0 \approx \omega_e$) as it is practically impossible to have RF pulses of sufficiently high amplitude to consider all spins on-resonance. As already pointed out in previous studies by Hurlimann and Song [25,26], each echo in the CPMG sequence can be decomposed to individual contributions of different coherence pathways. The number of coherence pathways contributing to an echo increases exponentially with the echo number $i$ ($i = 1, 2, 5, 13, 35, 96, 267, 750, \ldots 253,188,111$ with $i = 1, 2, 3, 4, 5, 6, 7, 8, \ldots 20$). Each pathway has its own $\Delta \omega_0/\omega_1$ = dependent contribution to the echo that becomes zero when $\Delta \omega_0/\omega_1$ = 0 except for the direct coherence pathway, which is then the only pathway contributing to the echoes. The relative contribution of the direct coherence pathway to the $i$th echo is given by

$$y_{\text{iMGSE},y} = \frac{S_{\text{iMGSE},0}(\Delta \omega_0/\omega_1, it)}{S_{\text{iMGSE},0}(0, it)} = \frac{1 - \cos(\pi/\sqrt{1 + (\Delta \omega_0/\omega_1)^2})}{2(1 + (\Delta \omega_0/\omega_1)^2)},$$

where it is assumed that RF pulses have ideal flip angles ($\omega_1\tau_{\text{RF}} = \pi/2$ for a 90° pulse and $\omega_1\tau_{\text{RF}} = \pi$ for a 180° pulse) [25]. Obviously, the contribution of the direct coherence pathway decreases with an increase of off-resonance $\Delta \omega_0/\omega_1$, and with the echo number $i$. For off-resonance spins in absence of diffusion and relaxation, it is remarkable that all echoes after first few initial ones are practically identical despite being composed of very different coherence pathways [27]. Echo amplitudes are affected only by off-resonance of the spins and not by the echo number. In presence of diffusion this is no longer true as echoes decay with the echo number. The decay is also faster with spins that are more off-resonance. Latter can be explained by different diffusion attenuations with different coherence pathways. Among all the pathways the direct pathway is least diffusion attenuated and for it, diffusion attenuation is given by Eqs. (3) and (4). For all other pathways, the diffusion attenuation is stronger so that echoes decay faster. As the contribution of these pathways becomes significant when $\Delta \omega_0 \approx \omega_1$, this also explains why the decay is faster with off-resonance spins than with on-resonance ones. For a general coherence pathway $q_1 \ldots q_i$ (to the $i$th echo) the diffusion attenuation is given by

$$y_{q_1 \ldots q_i} = \exp\left(-\frac{\eta_{q_1 \ldots q_i} G_j^2 T_{\text{MGSE}}^2}{12}\right).$$

Here $\eta_{q_1 \ldots q_i}$ denotes normalized diffusion decay rate [25,28], a parameter that controls the diffusion attenuation. The parameter is equal to one for the direct coherence pathway and is more than one for all the other pathways. Values of the parameter for all coherence pathways contributing to the first four echoes are given in Table 1. From the table it can be seen how the normalized diffusion decay rate parameter increases with more “complicated” pathways, i.e., the pathways that have number of instances in which magnetization is stored in the z-component (events denoted by “-” in the pathway notation) or have repeated events without magnetization sign change (several “+” or “-” signs in a row).

2.3. Diffusion probe

Experiments were performed with a home build diffusion probe (Fig. 1b). The probe uses a Maxwell pair type design of gradient coils; each coil of the pair has 75 turns of 0.3 mm thick copper wire in diameter of 2 cm, the coils are 1 cm apart. When fully loaded the gradient coil can produce a maximum magnetic field gradient of 6 T/m. In the middle of the gradient coil a 7 mm solenoid RF coil is placed with its axis perpendicular to the axis of the gradient coil. The diffusion probe has also thermocouple
(cupper-constantan) sensor installed to measure the gradient coil temperature. The probe is designed for installation in horizontal-bore magnets with its gradient direction equal to the static magnetic field direction. Samples for the probe are stored in specially designed small glass containers of a cylindrical shape with outer/inner diameter of 7/5 mm (2r = 5 mm) and of 10 mm length. The samples are inserted in the RF coil vertically so there is no danger of spill from the container in case of liquid samples.

2.4. NMR spectrometer

The diffusion probe was inserted in a 2.35 T (100 MHz proton frequency) horizontal bore superconducting magnet (Oxford Instruments, Abingdon, UK). The gradient coil was connected to a 10 A gradient amplifier (Bruker, Ettingen, Germany) and the RF coil to 300 W RF power amplifier (AMT, Brea, CA, USA) of which power was limited to 100 W in order to not overload the RF coil. The corresponding frequency of the excitation $B_1$ field was equal to $\frac{\nu}{2\pi} = 135$ kHz (calculated from $3.7 \mu$s duration for the 180° RF pulse). All the hardware components were controlled by an Apollo spectrometer (Tecmag, Houston, TX, USA).

2.5. Samples

Most of the study, i.e., testing of the effect of off-resonance spins on performance of the MGSE sequence, was performed on a distilled water sample. MGSE sequence with on-resonance spins was performed also on other samples including: mixture of distilled water (80%) and glycerin (20%), plant tissue sample represented by a piece $(5 \times 5 \times 10$ mm$^3$) of an apple (sort “Red Delicious”) and porous system made of closely packed 9–13 μm glass spheres (Sigma Aldrich, St. Louis, MO, USA) and admixed water. All samples were measured at room temperature of 23°C.

2.6. MGSE experiment

A MGSE experiment consisted of 120 runs of the MGSE sequence (Fig. 1a) with total number of spin-echoes in the sequence $N$ increasing in steps of 10 from 10 to 600 ($N = 10, 20 \ldots 600$). In all sequences $T_{MGSE} = 100$ ms so that according to Eq. (5) the corresponding MGSE frequencies, at which diffusion was measured, were equal to $\nu = 50, 100, \ldots 3000$ Hz. With each number of pulses $N$, the sequence was run twice to separate diffusion from relaxation signal contributions using Eq. (3), once with $G_N = N A G$ and once with $G_N = 0$. Parameter $A G = 7.9 \times 10^{-3}$ T/m was calculated from Eq. (7) using a predicted diffusion spectrum average value $D_p = 2.2 \times 10^{-9}$ m$^2$/s (diffusion of water at 23°C) and with parameter $f = 0.5$ (last echo in the MGSE train is one-half the amplitude of the first echo). In the MGSE sequence, phases of all refocusing RF pulses were equal and were shifted with respect to the excitation RF pulse by 90° (standard CPMG experiment); no phase cycling or signal averaging was used. Repetition time of the sequence was 10 s which yielded the total experiment time of 20 min. In the MGSE sequence, signal was acquired in every echo of the sequence in 128 acquisition points separated by dwell time of 1 μs, so that the echo acquisition time $t_{EA}$ was equal to 128 μs. The acquisition spectral bandwidth was equal to 1 MHz and acquisition filter also matched the spectral bandwidth. The acquired signals were processed in two ways differing in treatment of the echo signals $S(G_N, \nu) = S(0, \nu)$. In the first, the echo signals were equal to the echo amplitudes in the time domain, while in the second the echo signals were obtained in the frequency domain as central (or other specific) frequency points of the corresponding Fourier transformed time domain echo signals. The so determined echo signals of all the MGSE sequences were then analyzed by the diffusion model fitting procedure given by Eq. (6), a result of which was a diffusion spectrum $D(\nu)$.

In the study, the workflow for the MGSE experiment included initial sample preparation with diffusion probe parameter fine adjustments followed by a start of a home-written VBScript program (Microsoft, Redmond, WA, USA) within NTNMR programing environment (Tecmag, Houston, TX, USA) for automatic data acquisition and their processing. The script was run in a loop with steps corresponding to each of the measured MGSE frequencies. In each step the script calculated all needed MGSE sequence parameters, started data acquisition and analyzed the data by the fitting procedure given in Eq. (6). When the script was ended all MGSE data with all frequencies were measured and the corresponding diffusion spectrum was calculated.

3. Results

Sequences of normalized spin echoes $y_i/y_1$ acquired by the MGSE sequence with $N = 50, 100, 200, 400, 600$ for the water sample are shown in Fig. 2. Color coded images in the left column show the normalized echo signals in the frequency domain (vertical direction) as a function of echo number $i$ (horizontal direction). As the echoes were acquired in the presence of magnetic field gradient $G_N$ they also correspond to one dimensional images (1D image profiles) of the sample along the gradient direction. To the left of the images, the expected image profiles of the sample are drawn in blue. Widths of the profiles increase with $N$ due to the increasing gradients $G_N$. In the last sequence with $N = 600$, the expected profile width of 1007 kHz is practically identical to the signal acquisition bandwidth of 1 MHz, i.e., the expected profile occupies the entire field of view. However, the measured profiles (echoes in the frequency domain) are limited in bandwidth by the excitation bandwidth of the refocusing RF pulses, which was equal to $2/\nu_{100} = 2.37 \mu$s $= 540$ kHz (also equal to $4\nu/2\pi$). This also explains why widths of the measured profiles stopped increasing beyond $N = 320$. Off-resonance signals of the cylindrical sample of radius $r$ are therefore bound by the condition $|\Delta \nu_0| < \min (\nu_{G_N}/2, \nu_0)$.

A closer look at the echo sequence images (Fig. 2, left column) reveals that non-central (off-resonance) echo points decay with echo number faster than the central (on-resonance) point. The effect is better seen in Fig. 3, which shows the echo signal as a function of echo number $i$ for different off-resonances $\Delta \nu_0/\nu_0$ with $N = 400$ (different horizontal profiles along the echo sequence image). Fig. 3a shows dependence of an echo decay curve amplitude $|\Delta \nu_0| < \min (\nu_{G_N}/2, \nu_0)$.
amplitude of the on-resonance decay curve. Different decay curve amplitudes are a consequence of the sample geometry combined with off-resonance effects of the refocusing RF pulses. One dimensional images profiles of the sample along the gradient direction $z$ are proportional to $1/\sqrt{C_0(z/r)^2}$ (Fig. 1b). The coordinate $z$ and off-resonance $\Delta \omega_0$ are linked by the relation $\Delta \omega_0 = \gamma G_N z$ so that the off-resonance echo signal is limited by the condition

$$\tilde{y}_i/\tilde{y}_{\text{on resonance}} \leq \sqrt{1 - (\Delta \omega_0/(\gamma G_N))^2}. \quad (12)$$

Echo signal decay curves for the first eight off-resonances from Fig. 3a are normalized to the first echo and plotted in a logarithmic scale (Fig. 3b). The plots confirm that echo signals of off-resonance spins decay with echo number faster than the on-resonance spins. The difference is more apparent with higher off-resonances.

The last result clarifies the differences in decay rates between echo amplitudes in the time domain (red curve) and zero frequency filtered echo signals, i.e., zero frequency points of Fourier transformed echoes, (black curve) in right column graphs in Fig. 2. Only on-resonance spins from the 7.8 kHz wide central frequency band contribute significantly to the echo signal intensity.
quency bandwidth contribute signal to the black curve, while all spins with frequencies determined by Eq. (11) contribute signal to the red curve. The signal of off-resonance spins is also weighted by condition in Eq. (12). According to Eq. (9), the signal represented with the black curve is composed practically only from the direct coherence pathway signal. As evident from Eq. (10) and Table 1, among all coherence pathways the direct one is least diffusion attenuated and it decays the slowest. The signal represented with the red curve is composed also from signals of many other more “complicated” indirect coherence pathways that are more diffusion attenuated than the direct coherence pathway (prevailing at low off-resonances).

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Fig. 3. The echo image with $N = 400$ from Fig. 2 is analyzed by plotting horizontal intensity profiles, i.e., a spin-echo signal train, corresponding to different off-resonance conditions $\Delta \omega_0/\omega_1 = 0, 0.17, \ldots, 1.91$. It can be seen that a relative contribution of given off-resonance spins is decreasing with an increasing off-resonance (a) and that the spin-echo train decays faster when spins are more off-resonance (b). The first is a consequence of the sample shape, while the second has an origin in an increasing contribution of more “complicated” indirect coherence pathways that are more diffusion attenuated than the direct coherence pathway (prevailing at low off-resonances).

Plots in Fig. 4a show water diffusion spectra of spins with different off-resonances $\Delta \omega_0/\omega_1$. The spectra were calculated using Eq. (6) that is valid only for the direct coherence pathway. From the plots it can be seen that diffusion spectra are overestimated when spins are off-resonance. The error is bigger with higher off-resonances. In Fig. 4b diffusion spectra of water are calculated for the two different treatments of echo signals. The red graph corresponds to a diffusion spectrum calculated from echo signal amplitudes in the time domain (red curves in Fig. 2), while the black graph corresponds to a diffusion spectrum calculated from zero frequency filtered echo signals (black curves in Fig. 2). It can be seen that the red graph gives overestimated results for a diffusion spectrum that increases with the MGSE frequency on the account of expanding range of off-resonance components with faster decaying echo signals. The increasing trend of the diffusion spectrum with MGSE frequency increase slows down significantly at frequencies above 1500 Hz. The slowdown is associated with a discontinuation of the off-resonance range expansion with $N > 320$ on the account of the 540 kHz excitation bandwidth of the refocusing RF pulses (Eq. (11), Fig. 2). The black graph shows only a minor increase with MGSE frequency and yields a diffusion spectrum that is predicted by Fick’s diffusion model for water at 23°C.

Fig. 5 shows diffusion spectra measured by the MGSE sequence of four different samples. The spectra of different liquids (water, 

water-glycerin mixture) are both practically flat and differ only in magnitude. Almost identical property, apart from its lower diffusion start with frequencies below 500 Hz, has the spectrum of the biological tissue (apple). Much different to all the spectra is the spectrum of the porous system (mixture of water and glass spheres). The spectrum exhibits a significant dependence of a diffusion coefficient on MGSE frequency. It starts with a low diffusion coefficient \(0.5 \times 10^{-9} \text{m}^2/\text{s at } 500 \text{ Hz}\) that increases almost by a factor of three with the highest frequencies \(1.7 \times 10^{-9} \text{m}^2/\text{s at } 3000 \text{ Hz}\).

4. Discussion

Although the MGSE sequence excels in ability of diffusion spectrum measurements at high frequencies, its application is not trivial and can easily lead to results that can be misinterpreted by incorrectly attributing a MGSE sequence property to a sample property. This is especially true for the samples in which restricted diffusion causes a significant frequency dependence of diffusion spectra. In the study, it is shown that the origin of the possible misinterpretations of the MGSE spectra are off-resonance spins, which contribute to MGSE echo signals via “complicated” coherence pathways that are more diffusion attenuated than the direct coherence pathway of on-resonance spins. A MGSE echo train with such off-resonance contributions therefore decays faster than the on-resonance one and gives overestimated results for the diffusion spectrum. A solution to this problem is to eliminate all the off-resonance signals from the MGSE echoes and thus make the direct coherence pathway dominant.

In the study, prevalence of the direct coherence pathway in MGSE echoes was achieved by zero frequency filtering of the echo signals. This is different than the usual approach where the echo signal is treated as the time domain echo amplitude. The proposed zero frequency filtered approach is also equivalent to the integral of the time domain echo signal, where the signal is considered as a complex number. In an ideal case, only the direct coherence pathway is contributing to the MGSE echo train. According to Eq. (9) this is when \(\Delta \omega_0/\omega_1 = 0\), which implies that in MGSE the signal

![Fig. 4. Plots of diffusion spectra of water calculated from diffusion-attenuated spin-echo trains for MGSE frequencies in the range 0–3 kHz. Diffusion spectra in graph (a) are measured for different off-resonance spins \(\Delta \omega_0/\omega_1 = 0, 0.17, \ldots, 1.22\), while two diffusion spectra in graph (b) correspond to the spin-echo signals of the entire sample (red curve) and to the spin-echo signals only from on-resonance spins \(\Delta \omega_0/\omega_1 = 0\) (black curve). All spectra were calculated assuming spin-echo diffusion attenuation corresponding to the direct coherence pathway. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)
originates from infinitesimally narrow slice. As the signal would then be zero, the condition is not realistic and there must always be some small signal off-resonances in order to get a MGSE signal. Due to the limited echo acquisition time $t_{AQ}$, each point of the Fourier transformed echo signal has a frequency bandwidth of $1/t_{AQ}$. The result applies also to the zero frequency point, so that in our study, the zero frequency filtered echo signals had also some off-resonance components limited by $|\Delta \omega_0|/2\pi < 3.9$ kHz. In order to narrow the bandwidth of the zero frequency point, echo acquisition time would need to be increased. However, this would imply a reduction of the upper MGSE frequency limit down from 3 kHz. Namely, with the MGSE sequence at 3 kHz, the difference between $\tau = 167 \mu$s and $t_{AQ} = 128 \mu$s was only 39 $\mu$s. The difference was needed for filter adjustments in the spectrometer before each signal acquisition and could not be reduced much further. An alternative to zero frequency filtering could be also a narrow band excitation obtained by replacing square RF pulses with adiabatic RF pulses [29]. However, there are concerns that sufficiently low $|\Delta \omega_0|/\omega_0$ could not be obtained with the pulses. In addition, the theory of coherence pathways used in the study is valid for squared RF pulses and not for adiabatic RF pulses.

The MGSE zero frequency filtering approach is in its basic principle related to the PIETA approach [30] as the effect of a constant gradient, which obviously acts as a filter for “complicated” coherence pathways in MGSE, is mimicked in PIETA by incrementing the phase of every other refocusing RF pulse. In MGSE, the filtering is achieved by Fourier transforming the time dimension (signals of every echo) and selecting only zero frequency, while in PIETA the time dimension is preserved and filtering is achieved by Fourier transforming the phase dimension (phases of refocusing pulses), which is an additional dimension in PIETA in comparison to MGSE. An advantage of PIETA is also that for the filtering no magnetic field gradient is needed so that the acquired signals are not diffusion attenuated, however, the approach is more time consuming than the MGSE approach due the additional phase dimension.

Another factor that limited the upper MGSE frequency to 3 kHz was performance of the diffusion probe along with the gradient system. As follows from Eq. (7), in the MGSE sequence the gradient amplitude is proportional to the MGSE frequency and also depends on diffusion attenuation factor $f$ and MGSE train duration $T_{MGSE}$. In the study, the highest gradient was equal to 4.74 T/m (at 3 kHz, $N = 600$) which was 77.9% of the maximum gradient of 6.09 T/m. Higher gradients could cause problems associated with gradient underperformance, nonlinearity and reduced stability over $T_{MGSE}$ interval. The gradients can be reduced by increasing $f$ or by increasing $T_{MGSE}$. The former results in a lower diffusion attenuation of the MGSE echo train and therefore in a less accurately determined diffusion spectrum, while the latter has as a consequence a higher $T_2$ signal attenuation and noisier data that again result in a less accurately determined diffusion spectrum. In some materials, as for example porous materials, $T_2$ is short so that $T_{MGSE}$ cannot be further increased. Many other substances have also a slower diffusion rate than was used as the predicted one in the study ($D_p = 2.2 \cdot 10^{-9}$ m²/s). For these substances, MGSE gradients would need to be higher unless further increase of $T_{MGSE}$ is possible.

Zero frequency filtered echo signals with off-resonances in the range $|\Delta \omega_0|/2\pi < 3.9$ kHz result in a decrease of the direct coherence pathway proportion with echo number on the account of an increase of other coherence pathways. According to Eq. (9), for spins with $|\Delta \omega_0|/2\pi = \pm 3.9$ kHz, the ratio between the direct coherence pathway and other pathways at the end of MGSE sequence with $N = 50, 100, 200, 400, 600$ is equal to: 0.96, 0.92, 0.84, 0.70, 0.59. This effect could also explain a slight increase of the diffusion spectrum with frequency in water and in the water-glycerin mixture. More likely, the increase is a consequence of sample heating from the gradient coil. Due to the absence of gradient coil cooling, the gradient coil temperature was raising during the MGSE experiment as $\Delta T = 0, 0, 0.4, 0.7, 1.5$ °C with the MGSE frequency $\nu = 0.5, 1, 1.5, 2, 2.5, 3$ kHz. That there was no additional significant sample heating due to application of RF pulses in the MGSE sequence was confirmed by a test MGSE experiment in which the temperature sensor was immersed in the water sample. In the test experiment the measured sample temperatures were practically identical to those of the gradient coil. According to [31], temperature rise in water from 23 °C to 25.5 °C results in an increase of diffusion coefficient by 0.16 \cdot 10^{-9} \text{m}^2/\text{s}, which corresponds to the observed increase of the diffusion constant (from 2.1 \cdot 10^{-9} \text{m}^2/\text{s} at 100 Hz to 2.26 \cdot 10^{-9} \text{m}^2/\text{s} at 3 kHz). On the contrary, underperformance of the gradient system would lead to underestimated diffusion constants.

The off-resonance limit defined in Eq. (11) assumes that all signal arises only from the main excitation lobe of the refocusing RF pulses of the width $4\Delta \omega_0$. However, this is not entirely true, because the RF pulses have rectangular shape and their excitation profile is...
a sinc function with not only the main lobe but of the side lobes too. Presence of these lobes can be seen also in the echo sequence image with $N = 600$ in Fig. 2 as two dark blue horizontal bands aside the central main band.

In some of the plots in Fig. 2 (right column) a fast decrease in first few echo amplitudes in the time domain (red curve) can be seen. The transient behavior is analyzed in [27] where amplitudes of the first two echoes in presence of a constant magnetic field gradient were analyzed analytically. Results of the analysis confirmed up to 20% difference in amplitude between the first two echoes, depending on the inter-echo time.

Diffusion spectra of four different samples in Fig. 5 have in common a reduced accuracy at frequencies below 100 Hz and above 2 kHz. The first is on the account of echo train instability with low $N$ and long $TE$ [25]. It is also more difficult to control the output of gradient amplifiers at very low outputs. The lowest gradient was 0.08 T/m (at 50 Hz, $N = 10$) which was only 1.3% on the maximum output. In addition, in some samples internal gradients are present. These superimpose to the external gradients and make diffusion attenuation stronger. As the effect of the combined gradients on the attenuation is not linear but quadratic, the effect of internal gradients can only be partially excluded by the relaxation compensation procedure in Eq. (3). Origin of the reduced accuracy at higher MGSE frequencies is in increasingly thinner slices with higher frequencies from where zero frequency filtered echo signals originate. The porous sample has the noisiest diffusion spectrum among all the diffusion spectra. This is due to lowest amount of water in it. For the spectrum, measurements at frequencies below 400 Hz were unreliable because of the fast echo train decay. In the experiments, all MGSE controlling parameters ($f$, $T_{MGSE}$, $D_p$) were identical with all four samples. Better results could be obtained by optimization of the parameters for each sample separately.

5. Conclusion

The MGSE sequence enables diffusion spectra measurements at relatively high frequencies, however, MGSE implementation is not trivial and can lead to overestimated diffusion measurements. In the study, it is shown that the origin of these errors are off-resonance spins that contribute to MGSE echoes with signals having stronger diffusion attenuation. The effect can be greatly reduced by zero frequency filtering of the MGSE echo signals. The proposed method was tested on distilled water and few other samples. As the samples vary a lot in their diffusion and relaxation properties, the results of the method can be improved further by optimization of MGSE parameters specifically for each sample.

References
