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Acquisition of pure-phase diffusion spectra using oscillating-gradient spin echo

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Abstract

Oscillating-gradient spin echo (OGSE) diffusion experiments have long been used to measure the short-time apparent diffusion coefficient, $D_{app}(t)$, in the presence of restricted diffusion, as well as the spectrum of the slow-motion velocity autocorrelation function. In this work, we focus on two previously unexplored aspects of OGSE experiments: convection compensation and acquisition of pure-phase diffusion spectra in the presence of homonuclear scalar couplings. We demonstrate that convection compensation afforded by single-echo OGSE compares well with that in double-echo convection-compensated PGSE experiments. We also show that, in the presence of homonuclear scalar couplings, setting the OGSE echo time to $1/2J$ enables acquisition of pure-phase diffusion spectra and yields more reliable D estimates than mixed-phase PGSE or OGSE spectra. Pure-phase OGSE acquisition is also compatible with measurements of the apparent diffusion coefficient at an arbitrary diffusion time. These features of OGSE can be valuable in diffusion measurements of scalar-coupled small-molecule probes in cellular and other heterogeneous systems.

Keywords: Convection compensation; NMR diffusion and flow measurements; Modified-cosine gradient pulse; Apparent diffusion coefficient; Homonuclear scalar couplings

1. Introduction

Oscillating-gradient spin echo (OGSE; Fig. 1) can deliver a number of unique advantages for the measurement of diffusion and flow [1,2]. The diffusion-sensitive magnetization helix is present in OGSE during the two gradient pulses (σ), but not during the time interval between them ($\Delta - \sigma$). Therefore, unlike in the pulsed field-gradient spin echo (PGSE)[1] or stimulated echo (PGSTE) diffusion experiments [3], molecular displacement in OGSE is measured on the time scale of σ rather than Δ. This eliminates the necessity to include eddy-current recovery time in the diffusion interval, and thus enables the measurement of the apparent diffusion coefficient, $D_{app}(t)$, at shorter diffusion times than those accessible in PGSE or PGSTE measurements ($t < 5$ ms). The accessibility of the short-t regime makes OGSE an attractive option for studies of restricted diffusion [4–6], packed-bead flow [2], or intermediate chemical exchange [7,8]. OGSE also enables an easy and efficient control of the spectrum of the effective field gradient, $\boldsymbol{g}^*(\omega)$; this can be employed for probing the spectrum of the velocity autocorrelation function in the presence of slow motion or restricted diffusion [9–11]. Diffusion attenuation for the OGSE pulse sequence shown in Fig. 1 is given by

$$S(g) = S(0)\mathrm{e}^{-D\frac{3}{4\pi^2 n^2}\gamma^2 g^2 \sigma^3}, \quad (1)$$

where n is the (integer) number of full periods in the sinusoidal gradient pulse; D is the diffusion coefficient of the measured species; γ is the magnetogyric ratio; g

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[1] Abbreviations: cmc, critical micellization concentration; CTP, coherence transfer pathway; PFG, pulsed field gradient; (PG)SE, (pulsed-field gradient) spin echo; PGSEcc, convection-compensating double PGSE.

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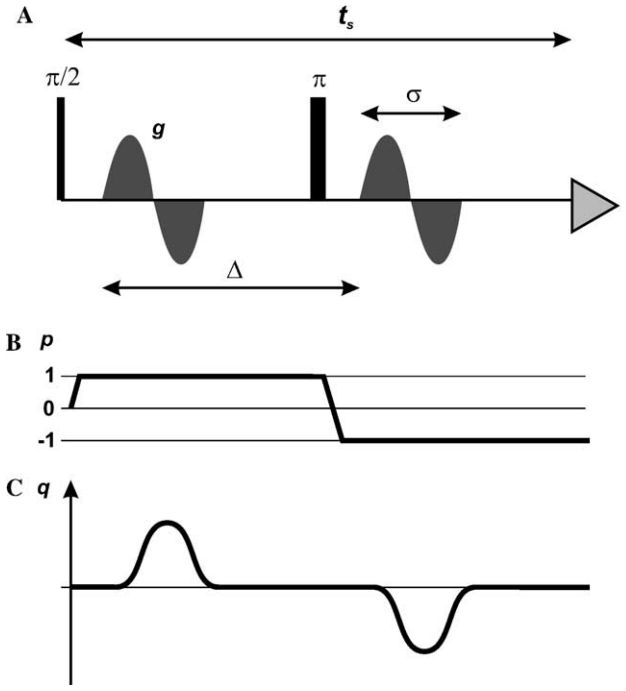

Fig. 1. OGSE diffusion measurement experiment: (A) the pulse sequence; (B) coherence transfer pathway; (C) evolution of the magnetization helix wave vector **q**. The magnitude of **q** is non-zero only while the gradients are switched on; therefore, the diffusion attenuation of the acquired signal is independent of the separation Δ between the gradient pulses.

is the field gradient amplitude; and σ is the full duration of the sinusoidal gradient pulse [2].

In this work, we focus on two aspects of OGSE which appear to have been either overlooked or not investigated in any detail. The first is its use for the measurement of the apparent diffusion coefficient in the presence of homonuclear scalar couplings. The knowledge of $D_{\mathrm{app}}(t)$ of scalar-coupled molecules is often required when studying chemical exchange and molecular transport of small-molecule probes in cellular suspensions [12,13]. A complication present in this type of measurements is that, if the diffusion interval Δ is not a multiple of $1/4J$, homonuclear scalar-coupled evolution produces a mixture of in-phase and anti-phase coherences at the beginning of acquisition. This can result in a distorted lineshape of the acquired spectrum, which in turn can adversely affect the estimate of the diffusion coefficient. Because in PGSE experiments the echo time Δ and the effective diffusion time t are mutually dependent ($t = \Delta - \delta/3$, where δ is the duration of the rectangular PGSE gradient pulses), pure-phase PGSE acquisition is not compatible with the use of an arbitrary diffusion time. In this work, we demonstrate that OGSE offers an additional degree of freedom in the choice of experimental parameters, because the spin–echo time can be set to $1/2J$ regardless of the value of the effective diffusion time. Therefore, OGSE enables the acquisition of pure-phase diffusion spectra at an arbitrary diffusion time.

The second focus of this paper is convection compensation in OGSE diffusion measurements. Convection compensation in PFG NMR is usually expressed by the condition

$$\int_0^{t_s} \mathbf{q}(t)\mathrm{d}t = 0, \tag{2}$$

where t_s is the duration of the pulse sequence (see Fig. 1). The quantity **q** is the wave vector of the diffusion-sensitive magnetization helix [3,14–16]

$$\mathbf{q}(t) = \int_0^t \gamma p(t')\mathbf{g}(t')\mathrm{d}t', \tag{3}$$

where p is the coherence order; the time dependence of p for the OGSE experiment is shown in Fig. 1B. The condition in Eq. (2) can be viewed as first-order compensation of flow, i.e., it enables the compensation for slow coherent motion (where the local velocities are approximately constant throughout t_s), but not necessarily for highly turbulent flow. In PGSE, a double echo is required to satisfy Eq. (2) [14,17,18]; **q** is positive during the first echo and negative during the second. In OGSE, the negative-**q** period is provided by the second gradient pulse during a single echo (see Fig. 1C), and no second echo is required to satisfy Eq. (2). Therefore, a single-echo OGSE pulse sequence is convection-compensated on the time scale of Δ.

Numerous modifications of the OGSE experiment can be envisaged; the respective formulae for diffusion attenuation can be obtained from the standard general expression [3]

$$S(q) = S(0)\mathrm{e}^{-D\int_0^{t_s} q^2(t)\mathrm{d}t}. \tag{4}$$

One possible modification is the use of a double-trapezoidal pulse, which is shown in Fig. 2A. This modification preserves all of the properties of OGSE described above, but provides for a greater diffusion attenuation of the NMR signal than that given in Eq. (1)

$$S(g) = S(0)\mathrm{e}^{-D\gamma^2 g^2\left[\frac{4}{3}\delta^3 + 2\delta^2\tau - \frac{1}{3}\delta\tau^2 + \frac{1}{15}\tau^3\right]}. \tag{5}$$

The meaning of δ and τ in Eq. (5) is evident from Fig. 2A. Another modification entails the use of gradient pulses which are compensated for convection on the time scale of σ. An example of this is shown in Fig. 2B: a cosine gradient pulse is modified to include a leading and a trailing ramp of duration τ, as well as a flat period τ in the middle of the pulse. The leading and the trailing ramps are used to avoid a sharp rise and fall of the gradient coil current; the flat period in the middle refocuses the phase imparted to spins by these ramps. This gradient pulse (called henceforth a modified-cosine pulse) is easily shown to be self-compensated for convection. Assuming a coherence order p and using Eq. (3), the time dependence of q is as follows:

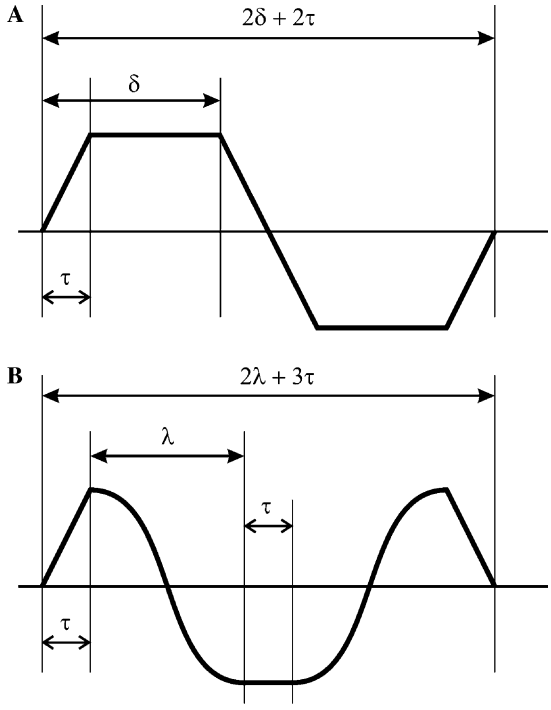

Fig. 2. Modifications of OGSE: (A) double-trapezoidal gradient pulse; (B) modified-cosine oscillating-gradient pulse. OGSE signal attenuation with the double-trapezoidal pulse is given by Eq. (5); with the modified-cosine pulse, Eq. (6) applies. The modified-cosine pulse consists of two half-period cosines (the two λ periods); leading and trailing ramps of duration τ to reduce eddy currents; and a flat τ period in the middle which compensates the **q** acquired by the magnetization during the ramp time. This gradient pulse is self-compensated for convection, i.e., the integral of **q** over the duration of the pulse is zero.

from 0 to τ	$p\gamma g t^2/2\tau$
τ to $\tau+\lambda$	$(p\gamma g/2)(\tau+2\lambda\sin[\pi(t-\tau)/\lambda]/\pi)$
$\tau+\lambda$ to $2\tau+\lambda$	$(p\gamma g/2)(\tau-2(t-\tau-\lambda))$
$2\tau+\lambda$ to $2\tau+2\lambda$	$-(p\gamma g/2)(\tau+2\lambda\sin[\pi(t-2\tau-\lambda)/\lambda]/\pi)$
$2\tau+2\lambda$ to $3\tau+2\lambda$	$-p\gamma g(t-3\tau-2\lambda)^2/2\tau$,

where $t = 0$ corresponds to the beginning of the gradient pulse, and the meaning of λ and τ is evident from Fig. 2B. Integration of $q(t)$ from $t = 0$ to $t = 2\lambda + 3\tau$ yields zero, which means that a single modified-cosine gradient pulse is convection-compensated in the sense of Eq. (2). For the OGSE pulse sequence with two modified-cosine gradient pulses, the diffusion attenuation of the signal is given by

$$S(g) = S(0)\mathrm{e}^{-D\gamma^2 g^2\left[\frac{2\lambda^3+8\lambda^2\tau}{\pi^2}+\lambda\tau^2+\frac{11}{30}\tau^3\right]}. \quad (6)$$

In this work, we measured the diffusion coefficient of propofol (structure shown in Fig. 3), a small molecule with multiple homonuclear scalar couplings [15,19], in two test systems using double-echo PGSE [18] and single-echo OGSE. The measurements were made in the presence of thermal convection. We demonstrate that the convection-compensating capacity of OGSE is at least as good as that of double-echo PGSE [18], and that both methods achieve their optimum performance when pure-phase spectra are acquired ($t_s = 1/J$ for in-phase; $t_s = 1/2J$ for antiphase).

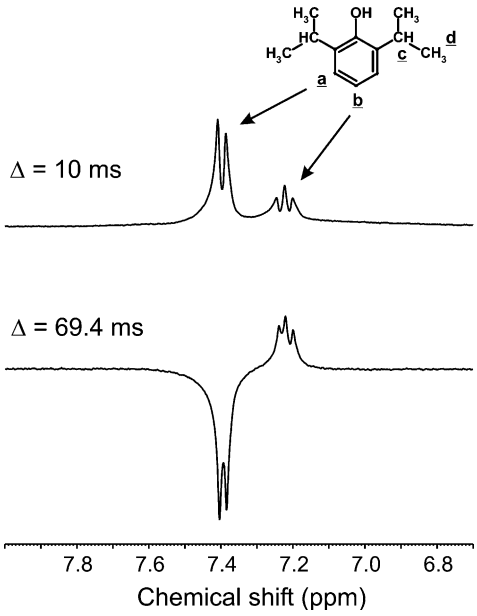

Fig. 3. Structure of propofol and representative OGSE diffusion spectra. These spectra are from propofol in sample **1** (1% propofol and 10% Solutol in D_2O–saline); both were recorded at $\sigma = 8$ ms and $g = 4.0\ \mathrm{T\ m^{-1}}$, but at different Δ values (shown in the Figure). In the spectrum with $\Delta = 10$ ms, the two multiplets are "sunken" in the middle, resulting in the relatively broad wings of the spectral peaks. This type of distortion was correlated with the overestimated D value of propofol (see Tables 1 and 2).

2. Materials and methods

2.1. Sample preparation

Reagents were purchased from the following sources: propofol, from Archimica SpA (Varese, Italy); Solutol HS15, from BASF (Ludwigshafen, Germany); CD_2Cl_2, from MSD Isotopes (Montreal, Canada); carbon tetrachloride (spectroscopic grade), from AJAX Chemicals (Auburn, NSW, Australia). All chemicals were used as received. Water was obtained from a Milli-Q reverse-osmosis apparatus (Millipore, Bedford, MA). The micellar solution of propofol [1% (w/w) propofol/10% (w/w) Solutol HS15/D_2O–saline] was prepared as described previously [15,19].

2.2. NMR setup and measurements

All measurements were carried out on a Bruker DRX-400 wide-bore NMR spectrometer equipped with a 1000 G cm^{-1} z-only actively shielded diffusion probe;

the general setup has been described previously [14,15,19,20]. Each sample was studied in a cylindrical Wilmad microcell (Buena, NJ) with an internal volume of 270 μL, outer diameter 8 mm, and nominal outer length 10 mm. This was inserted into a 10-mm NMR tube filled with CCl_4 for magnetic susceptibility matching. The length of the microcell enabled the sample to be contained within the constant-gradient region of the probe. The ramp times for trapezoidal and modified-cosine gradient pulses were 0.1 ms. No field-frequency lock was used. OGSE measurements were made with a multiple of four transients using EXORCYCLE phase cycling. NMR data were processed, and the diffusion coefficients determined, as described previously [14,19–21]. Phase correction of diffusion spectra was uniform within any given experimental set; baseline correction, where used, was linear. OGSE Stejskal-Tanner plots were processed according to Eqs. (1) and (6).

Table 1
Representative linewidths of aromatic protons of propofol in sample **1** at 38.0 ± 0.5 °C

Measurement	t_s (ms)	$\Delta\nu_{1/2}$ (Hz)	$\Delta\nu_{1/4}$ (Hz)	$\Delta\nu_{1/8}$ (Hz)
90°-acquire	0	15.6	25.9	42.6
		19.1	26.4	36.7
PGSEcc[a]	20.0	19.6	32.3	53.3
$\Delta = 5$ ms		25.4	45.5	>66[b]
Modified-Cos OGSE	24.0	18.6	29.3	55.3
$\Delta = 12$ ms		25.4	>47[b]	>60[b]
PGSEcc	138.8	14.7	24.9	39.6
$\Delta = 34.7$ ms		19.1	28.4	43.5
OGSE	138.8	15.2	24.9	39.1
$\Delta = 69.4$ ms		21.6	31.3	43.5

The two values in each cell refer to protons a and b (see Fig. 3). The columns $\Delta\nu_{1/2}$, $\Delta\nu_{1/4}$, and $\Delta\nu_{1/8}$ show the width of the multiplets at half, quarter, and one-eighth height, respectively.

[a] Abbreviations not defined in text: PGSEcc, convection-compensating double PGSE [18]; Modified-Cos OGSE, OGSE with modified-cosine gradient pulses (Fig. 2B).

[b] The two multiplets overlapped at or above the indicated level.

3. Results

Propofol was used as the test molecule in all of the experiments presented in this work. This choice was made because the molecule contains a number of distinct, homonuclear-coupled protons, and because its diffusion behaviour was studied by us previously [15,19]. Propofol is readily soluble in organic solvents and aqueous solutions of nonionic surfactants. In this work, the diffusion coefficient of propofol was measured in two test systems: (**1**) 1% (w/w) propofol and 10% (w/w) Solutol HS15 in D_2O–saline at 38.0 ± 0.5 °C; (**2**) 4.7% (w/w) solution of propofol in CD_2Cl_2, which was studied at two temperatures: 35.8 ± 0.5 and 37.4 ± 0.5 °C. The aspect ratios of the two samples were similar (sample inner height, 8 mm; inner cross-section, ~6.5 mm, as calculated from Wilmad's microcell specifications).

Propofol protons exhibited two three-bond homonuclear scalar couplings: $^3J_{ab} = 7.5$ Hz between aromatic protons a and b (see Fig. 3), and $^3J_{cd} = 6.8$ Hz between aliphatic protons c and d. The values of the J constants were measured from sample **2**. The average value of $1/2J$ for these two constants is 70 ms. The coupling constants observed in sample **1** had values similar to sample **2**, although the relatively large linewidths and spectral crowding made precise measurement in the former sample difficult. No coupling constants could be measured for Solutol protons.

The diffusion coefficient of propofol in sample **1** was measured from aromatic and methyl peaks and, when the quality of the spectra permitted, the isopropyl peak. Representative linewidths of aromatic propofol protons in this sample are shown in Table 1; diffusion coefficients of propofol and Solutol are presented in Tables 2 and 3, respectively. The composition of sample **1** and the conditions under which it was studied were identical to those used in previous work from this laboratory, and the results reported in Tables 2 and 3 can be directly compared to those reported in Table 3 of [15].

The spectra of sample **2** were considerably less crowded, and the diffusion coefficient was measured from each of the five observed peaks of propofol protons (including isopropyl and hydroxyl). Representative proton linewidths in this sample at 35.8 °C are shown in Table 4. The diffusion coefficients of propofol are presented in Tables 5 (35.8 °C) and Table 6 (37.4 °C); the D values within each cell are listed in the following order: a, b, OH, c, d.

Stejskal–Tanner plots obtained from convection-uncompensated PGSE experiments were slightly oscillatory and, in the case of sample **2**, curved upwards. The single-echo PGSE diffusion coefficients were therefore determined from the initial slopes of the plots.

4. Discussion

The values of propofol proton scalar coupling constants previously measured from a $CDCl_3$ solution were 7.6 Hz ($^3J_{ab}$) and 6.9 Hz ($^3J_{cd}$) [15,19]. The differences between these values and those measured from sample **2** are well within the digital resolution used (0.5 Hz).

Solutol is a nonionic surfactant which forms an isotropic micellar phase when dissolved in water: at 25 °C, its cmc is 0.21 mM or 0.02% (w/v) [22]. While no scalar couplings could be measured from the ^1H spectrum of Solutol in sample **1**, the structures of its chemical components suggest its protons should be subject to a complicated pattern of three-bond scalar couplings [19]. Propofol, which is very poorly soluble in water, is readily solubilized in a micellar aqueous solution of Solutol. In previous studies [19], most of the propofol in

Table 2
Diffusion coefficients of propofol in the Solutol HS15/D_2O solution (sample **1**) at 38.0 ± 0.5 °C measured by different ^1H NMR methods

Measurement	$\cos(\pi J t_s)$[a]	$D \times 10^{11}$ ($m^2 s^{-1}$)	Linear range
PGSE	0.90 (mixed-phase)	4.4 ± 0.2	0.3[b]
$\Delta = 10$ ms $\delta = 2$ ms $g \leqslant 6.4$ T m^{-1}			
PGSEcc[c]	0.90 (mixed-phase)	2.00 ± 0.01	1.0
$\Delta = 5$ ms $\delta = 2$ ms $g \leqslant 9.3$ T m^{-1}		2.06 ± 0.01	1.0
PGSEcc	−1.00 (in-phase)	1.92 ± 0.01	2.2
$\Delta = 34.7$ ms $\delta = 2$ ms $g \leqslant 4.6$ T m^{-1}		1.97 ± 0.01	1.8
		1.93 ± 0.01	2.5
OGSE	−1.00 (in-phase)	1.92 ± 0.01	1.5
$\Delta = 69.4$ ms $\sigma = 8$ ms $g \leqslant 9.3$ T m^{-1}		1.91 ± 0.02	1.6
		1.93 ± 0.01	1.9
OGSE	0.04 (antiphase)	1.94 ± 0.01	1.7
$\Delta = 34.7$ ms $\sigma = 8$ ms $g \leqslant 9.3$ T m^{-1}		1.88 ± 0.01	1.7
		1.94 ± 0.01	1.0
		1.86 ± 0.02	1.0
OGSE	0.90 (mixed-phase)	2.52 ± 0.03	1.8
$\Delta = 10$ ms $\sigma = 8$ ms $g \leqslant 9.3$ T m^{-1}		2.34 ± 0.03	2.1
OGSE, $n = 2$	−1.00 (in-phase)	1.95 ± 0.01	1.0
$\Delta = 69.4$ ms $\sigma = 10$ ms $g \leqslant 9.3$ T m^{-1}		1.88 ± 0.01	0.8
		1.96 ± 0.01	1.0
Modified-Cos OGSE[c]	−1.00 (in-phase)	1.92 ± 0.01	1.2
$\Delta = 69.4$ ms $2\lambda + 3\tau = 10$ ms $g \leqslant 9.3$ T m^{-1}		1.92 ± 0.02	1.0
		1.93 ± 0.01	1.2
Modified-Cos OGSE	0.04 (antiphase)	1.94 ± 0.01	1.2
$\Delta = 34.7$ ms $2\lambda + 3\tau = 10$ ms $g \leqslant 9.3$ T m^{-1}		1.93 ± 0.01	1.0
		1.93 ± 0.01	0.9
		1.98 ± 0.03	1.0
Modified-Cos OGSE	0.86 (mixed-phase)	2.56 ± 0.03	1.1
$\Delta = 12$ ms $2\lambda + 3\tau = 10$ ms $g \leqslant 9.3$ T m^{-1}		2.38 ± 0.03	1.3
Modified-Cos OGSE	0.94 (mixed-phase)	1.92 ± 0.03	0.2
$\Delta = 8$ ms $2\lambda + 3\tau = 6$ ms $g \leqslant 9.3$ T m^{-1}		2.19 ± 0.05	0.2

Multiple values in each cell refer to the different propofol signals. "Linear range" is the $\log_{10}$ vertical span of the Stejskal–Tanner region in which signal attenuation was linear [14,15].

[a] $J = 7$ Hz was used to calculate the values in this column.

[b] Convection oscillations were present; the initial slope of the Stejskal–Tanner plots was used for the determination of D in this measurement.

[c] Abbreviations not defined in the text: PGSEcc, convection-compensating double PGSE [18]; Modified-Cos OGSE, OGSE with modified-cosine gradient pulses (Fig. 2B).

this system was found to reside in the micelles; there was also a small extramicellar population. Chemical exchange between the two pools of propofol was estimated to be very rapid, and a single diffusion coefficient was observed. Due to the presence of an extramicellar population, the value of the diffusion coefficient of propofol was somewhat higher than the micellar D of Solutol [19,21].

PGSEcc at $\Delta = 34.7$ ms serves as a benchmark for the diffusion measurements performed on sample **1** (Tables 2 and 3). This experiment was done on the same system previously, and its results were found to be in good agreement with data from other PFG NMR diffusion techniques [15,19]. The average benchmark value of the diffusion coefficient of propofol in sample **1** was $(1.94 \pm 0.02) \times 10^{-11}$ $m^2 s^{-1}$. The micellar diffusion coefficient of Solutol obtained from the same measurement was $(1.67 \pm 0.01) \times 10^{-11}$ $m^2 s^{-1}$. The finding that D(Solutol) was lower than D(propofol) was consistent with the presence of extra-micellar propofol, the latter having a larger diffusion coefficient than propofol residing in Solutol micelles. The values of the diffusion coefficients obtained from single-echo PGSE measurements (PGSE, $\Delta = 10$ ms) were significantly larger than the benchmark values, and the Stejskal–Tanner plots were nonlinear. Therefore, convection in sample **1** was considered to be significant, and convection compensation was required even at short values of Δ.

The diffusion coefficients obtained from OGSE measurements at $\Delta = 34.7$ and 69.4 ms were in good agreement with the benchmark values: the average D values of propofol from these measurements were $(1.90 \pm 0.04) \times 10^{-11}$ and $(1.92 \pm 0.02) \times 10^{-11}$ $m^2 s^{-1}$, respectively. As expected from Eqs. (1) and (6), the relative OGSE signal attenuation was independent of Δ, and only the knowledge of the temporal and amplitude characteristics of the gradient pulses was required for the determination of the diffusion coefficients. Propofol multiplets in the diffusion spectra obtained at $\Delta = 69.4$ ms were in-phase (positive in-phase triplet, neg-

Table 3
Diffusion coefficients of micellar Solutol in the Solutol HS15/D_2O solution (sample **1**) at 38.0 ± 0.5 °C measured by different ^1H NMR methods

Measurement	$D \times 10^{11}$ ($m^2\ s^{-1}$)	Linear range
PGSE	4.6 ± 0.2	0.3
$\Delta = 10$ ms $\delta = 2$ ms $g \leqslant 6.4$ T m^{-1}	4.1 ± 0.2	0.3
PGSEcc	1.71 ± 0.01	1.2
$\Delta = 5$ ms $\delta = 2$ ms $g \leqslant 9.3$ T m^{-1}		
PGSEcc	1.67 ± 0.01	2.2
$\Delta = 34.7$ ms $\delta = 2$ ms $g \leqslant 4.6$ T m^{-1}	1.68 ± 0.01	1.9
OGSE	1.68 ± 0.01	1.5
$\Delta = 69.4$ ms $\sigma = 8$ ms $g \leqslant 9.3$ T m^{-1}	1.67 ± 0.01	1.6
OGSE	Not measured:	0
$\Delta = 34.7$ ms $\sigma = 8$ ms $g \leqslant 9.3$ T m^{-1}	mixed-phase Solutol peak	
OGSE	2.15 ± 0.03	1.5
$\Delta = 10$ ms $\sigma = 8$ ms $g \leqslant 9.3$ T m^{-1}	2.13 ± 0.03	2.1
Modified-Cos OGSE	1.70 ± 0.01	1.0
$\Delta = 69.4$ ms $2\lambda + 3\tau = 10$ ms $g \leqslant 9.3$ T m^{-1}	1.67 ± 0.01	1.0
Modified-Cos OGSE	2.13 ± 0.03	0.8
$\Delta = 12$ ms $2\lambda + 3\tau = 10$ ms $g \leqslant 9.3$ T m^{-1}		
Modified-Cos OGSE	1.91 ± 0.03	0.1
$\Delta = 8$ ms $2\lambda + 3\tau = 6$ ms $g \leqslant 9.3$ T m^{-1}		

Table 4
Representative linewidths of propofol protons in sample **2** at 35.8 ± 0.5 °C

Measurement	t_s (ms)	$\Delta\nu_{1/2}$ (Hz)	$\Delta\nu_{1/4}$ (Hz)	$\Delta\nu_{1/8}$ (Hz)
90°-acquire	0	11.7	16.6	24.9
		17.6	22.0	30.8
		4.9	9.3	16.6
Modified-Cos OGSE	12.0	13.2	19.1	28.4
$\Delta = 6$ ms		18.1	24.5	36.2
		4.9	9.8	16.6
PGSEcc	24.0	14.0	21.0	32.8
$\Delta = 6$ ms		19.1	28.4	>45[a]
		4.9	9.3	16.5
PGSEcc	138.8	12.2	17.5	25.4
$\Delta = 34.7$ ms		17.2	21.0	27.4
		5.0	9.3	16.1

The three values in each cell refer to protons a, b, and the hydroxyl proton (see Fig. 3).
[a] Two multiples overlapped at or above the indicated level.

ative in-phase doublet); those at $\Delta = 34.7$ ms were antiphase. Peak integrals of the antiphase OGSE spectra were highly reproducible, unlike the non-echo antiphase diffusion spectra based on a 3-pulse gradient-selected DQF COSY [15]. Solutol peaks in OGSE diffusion spectra at $\Delta = 35$ ms could be neither phased nor used for the determination of D. Their complicated mixed-phase structure indicated a composite nature of these peaks, which was consistent with the chemical structure of the main component of Solutol, poly(ethyleneglycol)(15) 12-hydroxystearate.

Table 5
Diffusion coefficients of propofol in CD_2Cl_2 (sample **2**) at 35.8 ± 0.5 °C measured by different ^1H NMR methods

Measurement	$D \times 10^9$ ($m^2\ s^{-1}$)	Linear range
PGSE	9.0 ± 0.5	0.8[a]
$\Delta = 6$ ms $\delta = 1$ ms $g \leqslant 2.4$ T m^{-1}		
PGSEcc	1.80 ± 0.01	2.3
$\Delta = 6$ ms $\delta = 1$ ms $g \leqslant 2.0$ T m^{-1}	1.80 ± 0.01	2.0
	1.79 ± 0.01	2.3
	1.77 ± 0.01	2.0
	1.77 ± 0.01	1.6
PGSEcc	1.89 ± 0.01	2.7
$\Delta = 34.7$ ms $\delta = 1$ ms $g \leqslant 1.0$ T m^{-1}	1.95 ± 0.02	2.3
	1.88 ± 0.02	2.4
	1.89 ± 0.01	2.4
	1.88 ± 0.01	2.8
OGSE	1.81 ± 0.01	2.0
$\Delta = 6$ ms $\sigma = 2$ ms $g \leqslant 7.8$ T m^{-1}	1.86 ± 0.01	1.4
	1.79 ± 0.01	1.8
	1.77 ± 0.01	1.5
	1.79 ± 0.01	1.8
OGSE	1.86 ± 0.01	2.0
$\Delta = 69.4$ ms $\sigma = 2$ ms $g \leqslant 7.8$ T m^{-1}	1.85 ± 0.01	2.0
	1.84 ± 0.01	1.8
	1.86 ± 0.01	1.8
	1.84 ± 0.01	1.8
Modified-Cos OGSE	1.86 ± 0.02	1.1
$\Delta = 6$ ms $2\lambda + 3\tau = 3$ ms $g \leqslant 8.8$ T m^{-1}	1.89 ± 0.03	1.1
	1.86 ± 0.02	1.1
	1.86 ± 0.02	1.1
	1.85 ± 0.02	1.1
Modified-Cos OGSE	1.86 ± 0.01	2.8
$\Delta = 69.4$ ms $2\lambda + 3\tau = 3$ ms $g \leqslant 8.8$ T m^{-1}	1.87 ± 0.01	2.4
	1.84 ± 0.01	2.0
	1.85 ± 0.01	2.7
	1.83 ± 0.01	2.7

The five values in each cell refer to different protons in the following order: a, b, OH, c, d (see Fig. 3).
[a] Proton a. The Stejskal–Tanner plots from this measurement were oscillatory; the initial slope was used for the determination of the diffusion coefficient.

The values of the measured diffusion coefficient of propofol at $\Delta = 5$–12 ms were significantly higher than those from the pure-phase OGSE or the benchmark PGSEcc measurements. This cannot be explained by convection effects, because the D values obtained at the short diffusion times ($\Delta = 5$–12 ms) consistently exceeded those obtained at $\Delta = 34.7$ or 69.4 ms, where convection could be expected to have a greater effect. The problem was not limited to OGSE experiments: PGSEcc measurement at $\Delta = 5$ ms also yielded slightly exaggerated D values. The second column of Table 2 shows that the overestimation of D in each measurement relative to the benchmark value correlated with the value of $\cos(\pi\ Jt_s)$. All of the pure-phase measurements (whether in-phase or antiphase) reproduced the benchmark D of propofol well, but none of the mixed-phase measurements did so. The bias of the measured D in mixed-phase measurements was unexpected. In terms

Table 6
Diffusion coefficients of propofol in CD_2Cl_2 (sample **2**) at 37.4 ± 0.5 °C measured by different ^{1}H NMR methods

Measurement	$D \times 10^9$ ($m^2\ s^{-1}$)	Change relative to Table 5 (%)	Linear range
PGSEcc	1.86 ± 0.01	+3.4	2.2
$\Delta = 6$ ms $\delta = 1$ ms	1.88 ± 0.01		2.2
$g \leqslant 2.0$ T m^{-1}	1.85 ± 0.01		2.3
	1.83 ± 0.01		2.2
	1.84 ± 0.01		2.2
PGSEcc	1.99 ± 0.01	+4.2	2.4
$\Delta = 34.7$ ms $\delta = 1$ ms	1.98 ± 0.01		2.3
$g \leqslant 1.0$ T m^{-1}	1.95 ± 0.01		2.2
	1.97 ± 0.01		2.4
	1.99 ± 0.01		3.9
OGSE	1.87 ± 0.01	+3.3	2.0
$\Delta = 6$ ms $\sigma = 2$ ms	1.88 ± 0.01		2.1
$g \leqslant 7.8$ T m^{-1}	1.85 ± 0.01		2.0
	1.84 ± 0.01		1.7
	1.87 ± 0.01		2.0
OGSE	1.91 ± 0.01	+2.7	2.1
$\Delta = 69.4$ ms $\sigma = 2$ ms	1.91 ± 0.01		2.0
$g \leqslant 7.8$ T m^{-1}	1.89 ± 0.01		2.0
	1.90 ± 0.01		2.1
	1.91 ± 0.01		2.1
Modified-Cos OGSE	1.93 ± 0.02	+5.4	2.9
$\Delta = 6$ ms $2\lambda + 3\tau = 3$ ms	2.05 ± 0.02		3.0
$g \leqslant 8.8$ T m^{-1}	1.92 ± 0.02		2.8
	1.99 ± 0.02		3.0
	1.92 ± 0.02		2.8
Modified-Cos OGSE	1.93 ± 0.01	+3.8	2.7
$\Delta = 69.4$ ms $2\lambda + 3\tau = 3$ ms	1.92 ± 0.01		2.3
$g \leqslant 8.8$ T m^{-1}	1.92 ± 0.01		2.3
	1.93 ± 0.01		2.3
	1.92 ± 0.01		2.9

of Eqs. (3) and (4), mixed-phase acquisition should not affect the measured diffusion coefficient, because the coherence order of both I_- and I_-S_z is -1. A likely explanation is apparent from Table 1, which shows representative linewidths of the aromatic multiplets of propofol in different experiments. The lines in the mixed-phase spectra ($t_s = 16$–24 ms) were significantly broadened compared to the lineshapes observed in non-echo spectra ($t_s = 0$); representative examples can be seen in Fig. 3. The broadening was either much smaller or absent in the pure-phase spectra. This is consistent with a previous observation that mixed-phase DQDiff acquisition can result in apparent baseline distortions of scalar-coupled multiplets [15]. These lineshape and baseline distortions have an adverse effect on the integration of spectral peaks and consequently on the estimates of the diffusion coefficients. Pure-phase acquisition, both in double-echo PGSE and single-echo OGSE measurements, eliminates these disadvantages.

Sample **2** was a simple solution of the hydrophobic propofol in a low-polarity solvent (CD_2Cl_2). Dichloromethane has a significantly lower viscosity than D_2O: for the protonated CH_2Cl_2, the value is 0.4 cP at 35 °C [23]; the boiling temperature of CH_2Cl_2 is 39.75 °C [24]. Therefore, we expect that thermal convection would have been more significant in sample **2** than in sample **1**; this is supported by the severely exaggerated value of D measured from non-convection compensated PGSE at $\Delta = 6$ ms (Table 5).

Unlike in sample **1**, the hydroxyl proton of propofol did not appear to be subject to chemical exchange in CD_2Cl_2: its NMR line was narrow, and there was no statistically significant difference between the D values obtained from the hydroxyl peak and the peaks of carbon-bound protons. The hydroxyl proton did not experience any three-bond scalar couplings, and no smaller couplings were apparent at the 0.5 Hz digital resolution. Therefore, although no benchmark D value was available for sample **2**, the hydroxyl peak enabled the elimination of scalar-coupling effects from the analysis of the propofol diffusion coefficient. At the same time, the diffusion coefficients measured from the other protons (aromatic, isopropyl, and methyl) were statistically indistinguishable from those obtained from the hydroxyl peak. The only significant exception from this appears to be the value determined from the aromatic triplet in the modified-cosine OGSE measurement with $\Delta = 6$ ms, $2\lambda + 3\tau = 3$ ms at 37.4 °C (Table 6, second line in the respective cell). This value was approximately 6% higher than those measured from other peaks under the same conditions; the aromatic triplet in the respective diffusion spectra showed slight broadening near the base (see Table 4). Overall, however, mixed-phase lineshape distortions were much smaller here than in sample **1**, and it appears that scalar couplings had almost no effect on the measured diffusion coefficients. Such differential effect between the two samples could be due to the presence of two populations of propofol in sample **1** or the relatively large viscosity of that sample.

Despite the apparent absence of the effects of mixed-phase acquisition, the diffusion coefficient values in sample **2** measured by different methods differed by up to 7% at each temperature studied: e.g., at 35.8 °C the range was between $(1.79 \pm 0.02) \times 10^{-9}$ $m^2\ s^{-1}$ (PGSEcc, $\Delta =$ 6 ms) and $(1.90 \pm 0.03) \times 10^{-9}$ $m^2\ s^{-1}$ (PGSEcc, $\Delta =$ 34.7 ms). For PGSEcc and sinusoidal-OGSE measurements, there was a loose positive correlation between t_s and the measured D: e.g., in OGSE measurements at 35.8 °C $D = (1.80 \pm 0.03) \times 10^{-9}$ $m^2\ s^{-1}$ at $\Delta = 6$ ms and $(1.85 \pm 0.02) \times 10^{-9}$ $m^2\ s^{-1}$ at $\Delta = 34.7$ ms. The results of the measurements performed at 37.4 °C formed a similar pattern. This correlation between the measured D and t_s was consistent with possible residual convection effects present in the long-Δ measurements. The modified-cosine OGSE measurements yielded D values which showed no significant dependence on t_s: e.g., $(1.86 \pm 0.03) \times 10^{-9}$ $m^2\ s^{-1}$ at $\Delta = 6$ ms and $(1.85 \pm 0.03) \times 10^{-9}$ $m^2\ s^{-1}$ at $\Delta = 34.7$ ms. This was consistent with the fact that in the modified-cosine OGSE

experiment convection was compensated on a shorter time scale (σ) than either in PGSEcc or sinusoidal-OGSE measurements (Δ). Nevertheless, a claim that the D variations seen in Tables 5 and 6 are due entirely to residual convection effects appears premature, and a more detailed study of the convection-compensating capacity of OGSE is needed. The use of mechanically driven flow (i.e., with a peristaltic pump) could be advantageous in such a study. Unlike thermally driven convection, mechanically driven flow would enable a high degree of control over the average flow speed; its use would also enable the measurement of unbiased diffusion coefficient at the same temperature at which the flow effects are studied.

OGSE diffusion measurements appear to be well suited for studying systems where the determination of the time-dependent apparent diffusion coefficient needs to be combined with pure-phase acquisition. Because of the total transverse evolution time of $1/J$, the technique should be useful primarily for small- to medium-sized molecules. Transmembrane transport of small, homonuclear scalar-coupled molecules in cellular suspensions appears to provide a useful prospective application of the technique.

5. Conclusions

In this work, we used double-echo PGSE and single-echo OGSE experiments to measure the diffusion coefficient of propofol in two test systems. The first test system was a micellar aqueous solution with a heterogeneous distribution of propofol between the micelles and the bulk aqueous phase, with very rapid chemical exchange between the two populations. The second system was a simple solution of propofol in an organic solvent of relatively low polarity and low viscosity. Both systems were studied in the presence of thermal convection. Single-echo OGSE provided adequate convection compensation. Pure-phase acquisition of diffusion spectra, achieved by setting the OGSE echo time to $1/2J$, yielded undistorted spectral lineshapes of propofol peaks and increased the reliability of the diffusion coefficient estimates. This advantage was particularly profound in the micellar solution; this could be attributed either to the heterogeneous distribution of propofol or the relatively slow rotational reorientation in this sample. Pure-phase OGSE acquisition is compatible with diffusion-time specific measurements of the apparent diffusion coefficient—a feature which is unavailable in PGSE or PGSTE experiments. Prospective applications of pure-phase OGSE experiments include diffusion of small-molecule probes in cellular suspensions, where the observed diffusion coefficient is time-dependent due to chemical exchange between extra- and intra-cellular compartments.

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References

[1] P.T. Callaghan, J. Stepisnik, Generalized analysis of motion using magnetic field gradients, Adv. Magn. Opt. Reson. 19 (1996) 325–388.
[2] M. Schachter, M.D. Does, A.W. Anderson, J.C. Gore, Measurements of restricted diffusion using an oscillating-gradient spin–echo sequence, J. Magn. Reson. 147 (2000) 232–237.
[3] C.S. Johnson, Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications, Prog. Nucl. Magn. Reson. Spectrosc. 34 (1999) 203–256.
[4] J.E. Tanner, Transient diffusion in a system partitioned by permeable barriers. Application to NMR measurements with a pulsed field gradient, J. Chem. Phys. 69 (1978) 1748–1754.
[5] P.N. Sen, Time-dependent diffusion coefficient as a probe of the permeability of the pore wall, J. Chem. Phys. 119 (2003) 9871–9876.
[6] P.P. Mitra, P.N. Sen, L.M. Schwartz, P. Ledoussal, Diffusion propagator as a probe of the structure of porous-media, Phys. Rev. Lett. 68 (1992) 3555–3558.
[7] J. Kärger, H. Pfeifer, W. Heink, Principles and application of self-diffusion measurements by nuclear magnetic resonance, Adv. Magn. Reson. 12 (1988) 1–89.
[8] A.R. Waldeck, P.W. Kuchel, A.J. Lennon, B.E. Chapman, NMR diffusion measurements to characterise membrane transport and solute binding, Prog. Nucl. Magn. Reson. Spectrosc. 30 (1997) 39–68.
[9] J. Stepisnik, Measuring and imaging of flow by NMR, Prog. Nucl. Magn. Reson. Spectrosc. 17 (1985) 187–209.
[10] C.S. Johnson, Diffusion measurements by magnetic field gradient methods, in: D.M. Grant, R.K. Harris (Eds.), Encyclopedia of Nuclear Magnetic Resonance, Wiley, New York, 1996, pp. 1626–1644.
[11] E.C. Parsons, M.D. Does, J.C. Gore, Modified oscillating-gradient pulses for direct sampling of the diffusion spectrum suitable for imaging sequences, Magn. Reson. Imaging 21 (2003) 279–285.
[12] K. Kirk, P.W. Kuchel, Equilibrium exchange of dimethyl methylphosphonate across the human red-cell membrane measured using NMR spin transfer, J. Magn. Reson. 68 (1986) 311–318.
[13] K. Kirk, P.W. Kuchel, Characterization of transmembrane chemical-shift differences in the P-31 NMR-spectra of various phosphoryl compounds added to erythrocyte suspensions, Biochemistry 27 (1988) 8795–8802.
[14] K.I. Momot, P.W. Kuchel, Convection-compensating PGSE experiment incorporating excitation-sculpting water suppression (CONVEX), J. Magn. Reson. 169 (2004) 92–101.
[15] K.I. Momot, P.W. Kuchel, Convection-compensating diffusion experiments with phase-sensitive double-quantum filtering, J. Magn. Reson. 174 (2005) 229–236.
[16] A. Jerschow, Thermal convection currents in NMR: flow profiles and implications for coherence pathway selection, J. Magn. Reson. 145 (2000) 125–131.
[17] A. Jerschow, N. Müller, Suppression of convection artifacts in stimulated-echo diffusion experiments. Double-stimulated-echo experiments, J. Magn. Reson. 125 (1997) 372–375.

[18] G.H. Sørland, J.G. Seland, J. Krane, H.W. Anthonsen, Improved convection compensating pulsed field gradient spin–echo and stimulated-echo methods, J. Magn. Reson. 142 (2000) 323–325.
[19] K.I. Momot, P.W. Kuchel, B.E. Chapman, P. Deo, D. Whittaker, NMR study of the association of propofol with nonionic surfactants, Langmuir 19 (2003) 2088–2095.
[20] K.I. Momot, P.W. Kuchel, D. Whittaker, Enhancement of Na^+ diffusion in a bicontinuous cubic phase by the ionophore monensin, Langmuir 20 (2004) 2660–2666.
[21] K.I. Momot, P.W. Kuchel, Pulsed field gradient nuclear magnetic resonance as a tool for studying drug delivery systems, Concepts Magn. Reson. 19A (2003) 51–64.
[22] K.-H. Frömming, C. Kraus, W. Mehnert, Physicochemical properties of the mixed micellar system of solutol HS15 and sodium deoxycholate, Acta Pharmaceut. Technol. 36 (1990) 214–220.
[23] D.W. Green (Ed.), Perry's Chemical Engineers' Handbook, seventh ed., McGraw-Hill, New York, 1997.
[24] M.J. O'Neil (Ed.), The Merck Index, thirteenth ed., Merck, New Jersey, 2001.