

The Use of Sample Rotation for Minimizing Convection Effects in Self-Diffusion NMR Measurements

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Undesirable temperature gradients in a NMR sample tube are usually generated by an inappropriate temperature regulation system. We have shown that such convection effects can greatly distort the measurement of translational self-diffusion coefficients. The use of sample spinning helps to minimize such undesirable effects by disruption of convection fluxes due to resulting Coriolis forces that have a strongly stabilizing effect on the conducting state of the system (J. Lounila *et al.*, *J. Magn. Reson. A* 118, 50 (1996)). This simple trick allows the accurate measurement of diffusion coefficients for a wide range of temperatures and solvents without the need for a convection-compensated NMR pulse sequences or more sophisticated temperature control units. Experimental data obtained for some target compounds dissolved in several common deuterated solvents at different temperatures are reported and discussed. © 2001 Academic Press

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INTRODUCTION

The measurement of translational self-diffusion coefficients (D) by high-resolution NMR (1–3) has become a helpful and powerful tool to analyze complicated mixtures without the need for previous chemical separation (4–5) and also to study intermolecular ligand–receptor interactions in, for instance, aggregation states (6–9), supramolecular complexes (10–12), or intermolecular hydrogen bonding (13). The idea of translational self-diffusion relies in a simple principle: small molecules moving quickly in solution present large coefficient values and their NMR resonances appear partially or fully attenuated when compared to those of larger molecules moving more slowly. Thus, the experimental D values are readily related to interesting molecular constraints, such as molecular size, molecular weight, and hydrodynamic radius.

Diffusion coefficients are experimentally determined by monitoring the signal intensity decay in a pulsed-field gradient spin-echo experiment spectrum as a function of the overall gradient strength (14). Recently, a comparative work on several available pulse sequences has been reported (15). Although all these NMR experiments are easily recorded and the corresponding data can be extracted and qualitatively analyzed with relative simplicity, the major challenge of these measurements is the precision with which the data are obtained. Two major sources of possible errors are due to eddy currents and convection effects. Eddy currents generated after the application of a pulsed-field gradient (PFG) are efficiently minimized using satisfactory recovery delays or appropriate NMR pulse sequences involving bipolar gradients, such as the widely accepted bipolar-gradient LED (BPLED) pulse sequence (16).

However, during our routine work we obtained anomalous results when measuring diffusion coefficients under standard laboratory conditions, basically attributed to the already described convection effects (17–23) arising from the presence of temperature gradients along the z -axis in the NMR sample tube. Temperature gradients are inherent to the available temperature regulation system incorporated in the default configuration of commercial spectrometers. Thus, when performing temperature-regulated NMR experiments, both air heating or nitrogen-gas cooling procedures of the NMR sample are usually applied from the bottom part of the probe and, therefore, unavoidable temperature gradients can be generated in the NMR tube arising for nonuniform heating/cooling of the sample. The severity of these temperature gradients greatly depends upon the efficiency of heat transfer and viscosity of the sample as well as other experimental factors such as gas flow rate, sample geometry, sample size, or probe design.

Experimentally, convection results in undesirable signal attenuation and, therefore, increased self-diffusion coefficients are erroneously measured. In order to check the experimental variables affecting such measurements, we measured the D value for several target compounds dissolved in typical deuterated

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solvents at different temperatures. The first goal of this work was to detect to what extent such convection effects could affect such experimental measurements as a function of the chosen solvent and temperature. We have shown here that such convection effects are usually present under standard conditions when temperature regulation is required and they must be detected and minimized when accurate measurements of self-diffusion coefficients are to be made.

The proposal of tricks and methods describing how we can avoid or minimize the deleterious effects of convection is an interesting topic for those interested in achieving such measurements with minimal errors and high accuracy under routine conditions. Recently, methods to measure convection effects and temperature profiles in liquid samples have been reported (17, 21). Practical approaches to minimize the effect of temperature gradients include the use of compensated pulses sequences (18, 19, 22), the use of a plunger (8), the decrease in sample tube diameter or/and the sample height, the increase in sample viscosity, the redesigning of the probe temperature control system, and the increase in the gas flow rate (17).

In modern high-resolution NMR experiments it is general practice to record experiments without sample spinning in order to minimize possible vibrational instabilities, spinning sidebands, background gradients, and/or subtraction artifacts that would affect the spectral quality. However, it was already proposed that convection artifacts could be simply removed by sample rotation (20). This approach was verified on inversion-recovery experiments applied on ^{129}Xe NMR of xenon gas and on ^{19}F NMR in xenon difluoride dissolved in deuterated organic solvents such as cyclohexane and acetonitrile (20). The basic of this surprising effect relays the resulting Coriolis forces that have a strongly stabilizing effect on the conducting effect of the system. On the other hand, a computer-controlled, stop-and-go spinner system applied only during the acquisition period was also proposed to enhance resolution and minimize signal overlapping from different species in diffusion experiments (24, 25). We have also evaluated the effect of sample rotation through all pulse sequences in the measurement of diffusion coefficients and the results for a series of solvent and temperature conditions are presented and analyzed.

EXPERIMENTAL

All NMR experiments described in this work were carried out on a 500-MHz AVANCE spectrometer equipped with a 5-mm, triple-resonance inverse, broadband probe head and a z -gradient coil controlled by a B-AFPA10 gradient unit. However, the general effects of convection described here were also observed and verified in a number of different spectrometers from our NMR laboratories and elsewhere equipped with different configurations.

In a diffusion NMR experiment using sinusoidal shaped gradients, the signal intensity of a given resonance decays as given

in Eq. [1]

$$A_g = A_o \exp(-\gamma^2 g^2 \delta^2 (4\Delta - \delta) D), \quad [1]$$

where A_g and A_o are the signal intensities in the presence and absence of PFG, respectively, γ is the gyromagnetic ratio (rad s G^{-1}), g is the strength of the diffusion gradients (G cm^{-1}), D is the diffusion coefficient of the observed spins ($\text{cm}^2 \text{s}^{-1}$), δ is the length of the diffusion gradient (s), and Δ is the time separation between the leading edges of the two diffusion pulsed gradients (s). Diffusion coefficients were further obtained by measuring the slope in the following linear relationship:

$$\ln(A_g/A_o) = -\gamma^2 g^2 \delta^2 (4\Delta - \delta) D. \quad [2]$$

The measurement of D was made using the bipolar-gradient LED (16) and the compensated BPLED (19) pulse sequences, using a diffusion time of 150 ms and a LED delay of 50 ms. In the case of $\text{H}_2\text{O}/\text{D}_2\text{O}$ solutions, a W5 block (27) was introduced in both sequences prior to acquisition for efficient solvent suppression. For each experiment, sine-shaped PFGs, with a duration of 1.5 ms followed by a recovery delay of 100 μs were incremented from 5 to 100% of the maximum strength in eight spaced steps. The strength of the gradient was first calibrated by measuring the self-diffusion coefficient of the residual HDO signal in a 100% D_2O sample at 298 K. A value of $1.90 \times 10^{-9} \text{ m}^2 \text{s}^{-1}$ was used for back calculation of the maximum gradient strength. All experimental data are the average result of three independent measurements.

The temperature regulation system eurotherm VT-3000 supplied by the manufacturer was used. Temperature calibration on the spectrometer was performed using a standard methanol sample. In all experiments requiring temperature regulation, the temperature was stabilized into a range of $\pm 0.1 \text{ K}$ using an air flow rate of 400 L/h. In room temperature experiments, maximum oscillations of $\pm 0.2 \text{ K}$ were permitted. Sample spinning of 20 Hz was used except when otherwise indicated.

Strychnine, menthone, β -cyclodextrin, and all deuterated solvents with normal purity were used as commercially available. Samples were prepared by dissolving the solute in ca. 0.7 mL of the corresponding deuterated solvent. Standard BRUKER samples of 2 mM-lysozyme in 90% $\text{H}_2\text{O}/10\% \text{ D}_2\text{O}$ and 80% glycol in DMSO were also used. 1D GROESY experiments were recorded as reported previously (29). A 20-ms 1% truncated Gaussian shaped pulse for selective inversion and a 3-KHz CW period for ROESY transfer were used. Gradients were optimized to a 1–1 : 2 ratio.

RESULTS AND DISCUSSION

Figure 1 shows a graphical display of the signal decay for a strychnine sample dissolved in CDCl_3 at several temperatures. In

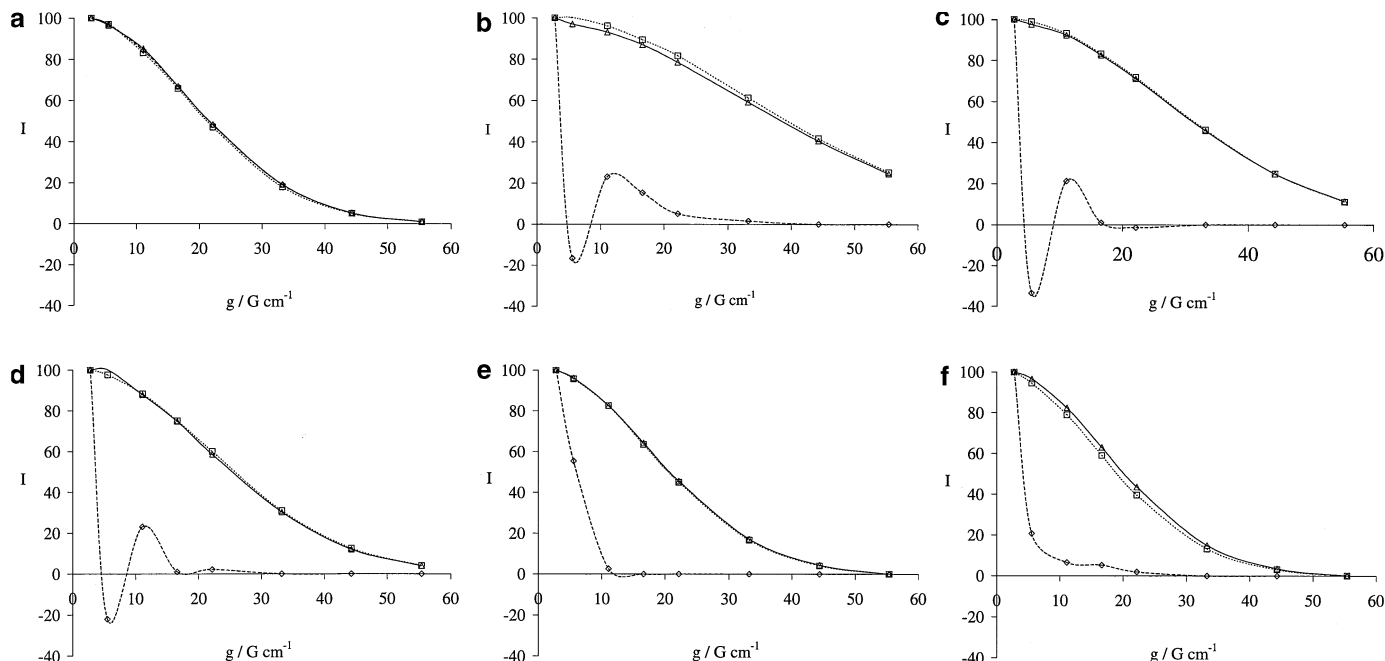


FIG. 1. Experimental intensity decay for a sample of strychnine dissolved in CDCl_3 at several temperatures: (a) room temperature, 295.5 K (heater off), (b) 233 K, (c) 253 K, (d) 273 K, (e) 298 K, and (f) 303 K. Each plot represents three different data: (---◇---) BPLED experiment without rotation, (.....□.....) BPLED experiment with rotation, and (—△—) compensated-BPLED sequence.

each graph, signal intensity is plotted vs gradient strength from which a theoretical exponential decay might result accordingly to Eq. [1]. Three different data are reported in each case, the first two acquired with the BPLED sequence with and without sample rotation and the third acquired with the compensated-BPLED sequence without sample rotation. Figure 1a is the measurement performed at room temperature (295.5) with the temperature control unit (TCU) switched off. It can be observed that all three measurements give comparable results, confirming the absence of almost no convection effect in CDCl_3 at room temperature, when there is no temperature regulation. However, Figs. 1b–1d show the same measurements performed at lower temperatures in which nitrogen gas cooling was applied. In all three cases it can be observed that strong convection effects are present when the measurement is performed without sample spinning. The results obtained under sample rotation were identical to those obtained using the compensated sequence. The same is true when heating the sample at 298 and 303 K (see Figs. 1e and 1f). In addition, more signal decay is observed when increasing the temperature. As deduced from Fig. 1, convection effects result in a faster decay of the signal intensity, giving erroneous and large coefficient values (see Table 1). As an experimental evidence for the existence of severe undesired convections effects, the signal intensity shows a strong oscillation in the signal amplitude when the intensity approaches the null point, giving in some cases even negative amplitude. In many cases, such maladjustments make it impossible to measure a reliable coefficient value, and this lack of linear behavior is reflected in

Tables 1 and 2 as undetermined data. The room temperature value without spinning correlates perfectly with the regulated temperature data measured with spinning and with the compensated sequence.

In order to check such effects under several experimental conditions we have performed the same measurements at several temperatures for a series of menthone samples dissolved in several common deuterated solvents, namely CDCl_3 , acetone, methanol, acetonitrile, toluene, benzene, and DMSO (Table 2 and Fig. 2). Several general trends can be observed: (i) In all cases, no convection effects were observed when measurements were made at room temperature, without temperature regulation; (ii) convection effects were detected to some extent for

TABLE 1
Translational Self-Diffusion Coefficients of Strychnine in CDCl_3 at Several Temperatures

Temperature (K)	BPLED (without) $D/10^{-5} \text{ cm}^2 \text{ s}^{-1}$	BPLED (with) $D/10^{-5} \text{ cm}^2 \text{ s}^{-1}$	Compensated-BPLED $D/10^{-5} \text{ cm}^2 \text{ s}^{-1}$
233	nd ^a	0.26	0.26
253	nd ^a	0.41	0.41
273	nd ^a	0.61	0.61
Room temperature (295.5)	0.88	0.89	0.87
298	nd ^a	0.94	0.92
303	nd ^a	1.03	0.99

^a nd, not determined due to strong decay.

TABLE 2

Translational Self-Diffusion Coefficients of Menthone Dissolved in Several Solvents at Different Temperatures

Temperature (K)	Solvent	BPLED (without) $D/10^{-5}$ $\text{cm}^2 \text{s}^{-1}$	BPLED (with) $D/10^{-5}$ $\text{cm}^2 \text{s}^{-1}$	Compensated-BPLED $D/10^{-5}$ $\text{cm}^2 \text{s}^{-1}$
273	CDCl_3	nd ^a	0.96	0.98
298	CDCl_3	nd ^a	1.46	1.47
303	CDCl_3	nd ^a	1.59	1.59
273	CD_3OD	nd ^a	0.97	1.02
298	CD_3OD	nd ^a	1.56	1.56
303	CD_3OD	nd ^a	1.62	1.69
273	CD_3CN	nd ^a	1.66	1.66
298	CD_3CN	nd ^a	2.44	2.46
303	CD_3CN	nd ^a	2.58	2.63
298	Toluene	nd ^a	1.61	1.62
303	Toluene	nd ^a	1.73	1.72
308	Toluene	3.36	1.86	1.84
298	Benzene	1.96	1.46	1.53
303	Benzene	nd ^a	1.57	1.63
308	Benzene	nd ^a	1.80	1.81
298	Acetone	nd ^a	2.63	2.61
303	Acetone	nd ^a	2.82	2.80
298	DMSO	0.49	0.50	0.50
303	DMSO	nd ^a	0.57	0.56
308	DMSO	nd ^a	0.64	0.62
295.3 (off)	CDCl_3	1.41	1.42	1.41
294.8 (off)	CD_3CN	2.30	2.32	2.31
294.8 (off)	Acetone	2.48	2.50	2.47
294.6 (off)	MeOH	1.47	1.56	1.46
294.8 (off)	Benzene	1.41	1.42	1.42
294.8 (off)	Toluene	1.51	1.52	1.50
292.2 (off)	DMSO	0.46	0.44	0.46

^a nd, not determined due to strong decay.

nonspinning samples. These effects were more pronounced in low-viscous solvents such as chloroform or acetone and they were also observed even for toluene and benzene. Figure 2 shows for comparison the signal decay in several solvents at 298 K; (iii) in the case of DMSO, convection effects were not observed at 298 K but they started to be noticeable at 303 K; (iv) data from sample spinning and from the compensated sequence give similar results within the experimental error, thus confirming the lack of undesired effects when rotation was performed.

A simple trick to check if the temperature regulation system installed in any NMR spectrometer causes undesired convection effects in an NMR sample consists of performing two different measurements, one working at room temperature with the TCU switched off and the other at a stabilized and controlled temperature close to room temperature with the TCU switched on. On the other hand, comparison of data with and without sample rotation also will give this information. For instance, in our spectrometer, no convection effects were detected for a CDCl_3 sample with the unit off at 295–296 K (without temperature control) but strong effects were present at 298 K with regulation that can be avoided by spinning the sample.

As already known, convection affects all compounds present in the solution in the same way and therefore these effects are also observed when monitoring the solvent signal in each case (data not shown). Furthermore, a good direct relationship between the experimentally measured coefficient and the viscosity of the deuterated solvent is accomplished (30).

In addition to the already demonstrated fact that sample rotation does not entail other undesirable effects on the experimental determination of diffusion coefficients, sample rotation improves line resolution (24). However, it is highly recommended to use high-quality NMR tubes in order to maximize the spinning speed stability and minimize the artifacts and undesirable sidebands that can be generated by sample rotation.

In order to check the extension of convection effects to other more viscous solvents, such as D_2O and DMSO solutions, we have also measured the translational self-diffusion coefficients for a sample of 10-mM β -cyclodextrin in 100% D_2O at several temperatures. Under such conditions, convection effects begin to appear at 303 K. Dramatic results are obtained at higher temperatures as clearly illustrated in Fig. 3, in which the dependence of $\ln(A/A_0)$ is represented as a function of the use of sample rotation and temperature.

When working with a sample 80% glycol in DMSO, convection effects appear near 308 K and, under extreme conditions (347 K), sample rotation does not completely compensate for the undesirable convection effects. We have experimentally found that increasing the spinning rate to 40 Hz alleviates such a deviation but some effects remain. Using the BPLED with spinning at 330 K, a value of $4.22 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ was obtained whereas an erroneous value of $9.4 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ was obtained without spinning. By comparison, Muller *et al.* (19) reported a value of 4.19×10^{-6} and $4.37 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ when measuring with a compensated STE and LED, respectively, under the same conditions.

Although it is clear that the compensated NMR pulse sequence works properly for small molecules, it can suffer from a series of drawbacks: (i) It is more sensitive to improper tuning/matching and also to pulse miscalibrations, (ii) a double phase cycled is needed, requiring a minimum of 32 steps to be recorded, thus increasing the total acquisition time, and (iii) it is unsuitable for use with large molecules, such as proteins, nucleic acids, or biomolecular complexes, having short T_2 relaxation times. In our hands, strong signal attenuation due to the fast transverse relaxation was observed when applied to a 2-mM lysozyme in a 90% $\text{H}_2\text{O}/10\% \text{D}_2\text{O}$ sample. Although water diffuses very quickly compared to larger molecules such as proteins, its strong resonance is usually a nuisance in diffusion NMR measurements and it is not perfectly suppressed. For this reason we have modified the BPLED and compensated BPLED pulse sequences by appending an excitation sculpting block (26) with a W5 pulse train (27) after the LED delay and just before acquisition in order to suppress the strong water resonance. The resulting BPLED-W5 spectra offer perfect solvent suppression, excellent baseline properties, and acceptable signal-to-noise ratios in a reasonable

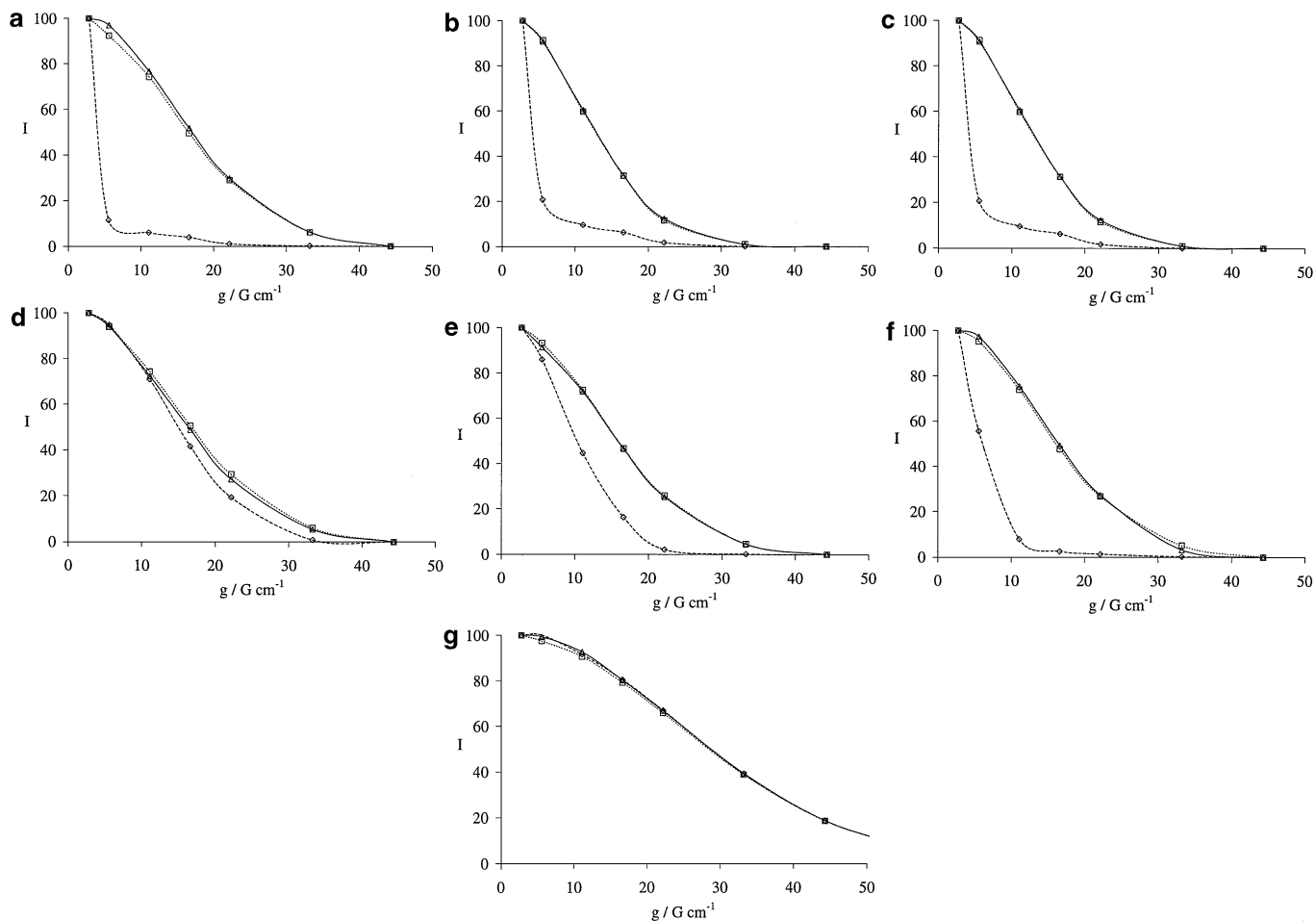


FIG. 2. Experimental intensity decay for a sample of menthone dissolved in several deuterated solvents at 298 K: (a) CDCl_3 , (b) acetone, (c) acetonitrile, (d) benzene, (e) toluene, (f) methanol, and (g) DMSO. Each plot represents three different data: (---- \diamond ----) BPLED experiment without rotation, (..... \square) BPLED experiment with rotation, and (— \triangle —) compensated-BPLED sequence.

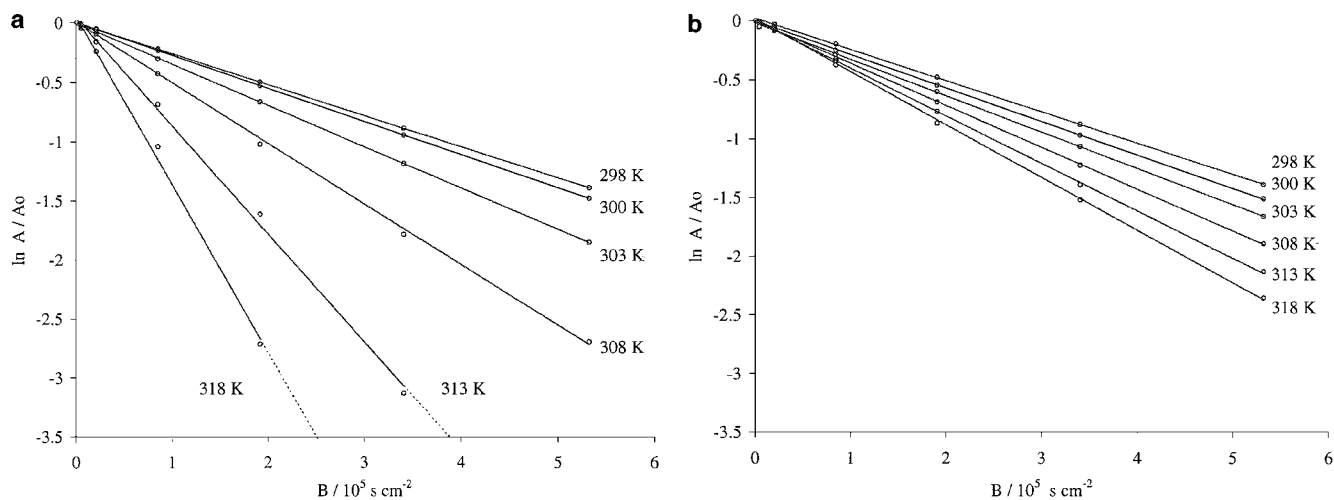


FIG. 3. Plot of $\ln(A/A_0)$ vs $B(\gamma^2 g^2 \delta^2 (4\Delta - \delta))$ for β -cyclodextrin in D_2O at several temperatures (a) without and (b) with sample spinning.

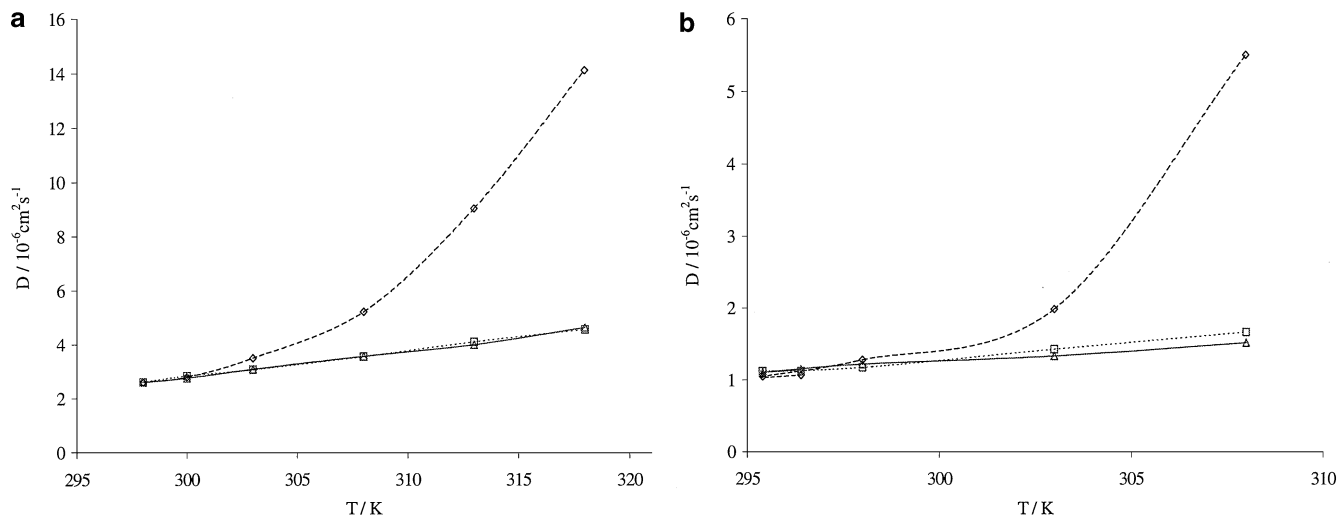


FIG. 4. Temperature dependence of the diffusion coefficients of (a) β -cyclodextrin in D_2O and (b) lysozyme in H_2O/D_2O with and without sample spinning. Three different data are represented: (---◇---) BPLED experiment without rotation, (·····□·····) BPLED experiment with rotation, and (—△—) compensated-BPLED sequence.

time period, which allows automated phase correction and accurate signal and/or area determinations in a simple routine way. However, in the case of the compensated BPLED-W5 sequence, strong signal attenuation is achieved giving critical signal-to-noise ratios that make the diffusion coefficient measurement difficult. Figure 4 clearly demonstrates that convection is present below 300 K and our experimental results agree with those previously reported (9).

Convection in NOE Experiments

Convection effects can be also a nuisance in any NMR experiment that use PFGs for coherence selection, in particular in all experiments where defocusing/refocusing PFGs are well separated in time. For instance, compensated pulse sequences for the original selective 1D NOESY or GOESY (28) and selective 1D ROESY or GROESY (29) experiments have been also proposed (18) to avoid such effects in gradient-selected selective 1D NOESY and ROESY experiments. In GOESY and GROESY experiments, ultra-clean 1D spectra are obtained because no difference spectroscopy is involved, at the expense of a sensitivity loss by a factor of 2 due to coherence selection. Sample rotation achieves the same goal using standard pulse sequences under temperature regulation. Figure 5 shows typical GROESY spectra applied on the H15a of strychnine in $CDCl_3$ at room temperature and a regulated 298 K. Clean spectra with maximum sensitivity and improved resolution are obtained when spinning is applied (Fig. 5b), whereas tremendous attenuation is observed when no sample rotation is applied (Fig. 5d). In addition, convection effects are more pronounced for stronger gradients and therefore it is highly desirable to use the minimum gradient strength in these experiments for a proper coherence selection. Another major effect of convection in NOE measurements is the shifting of the

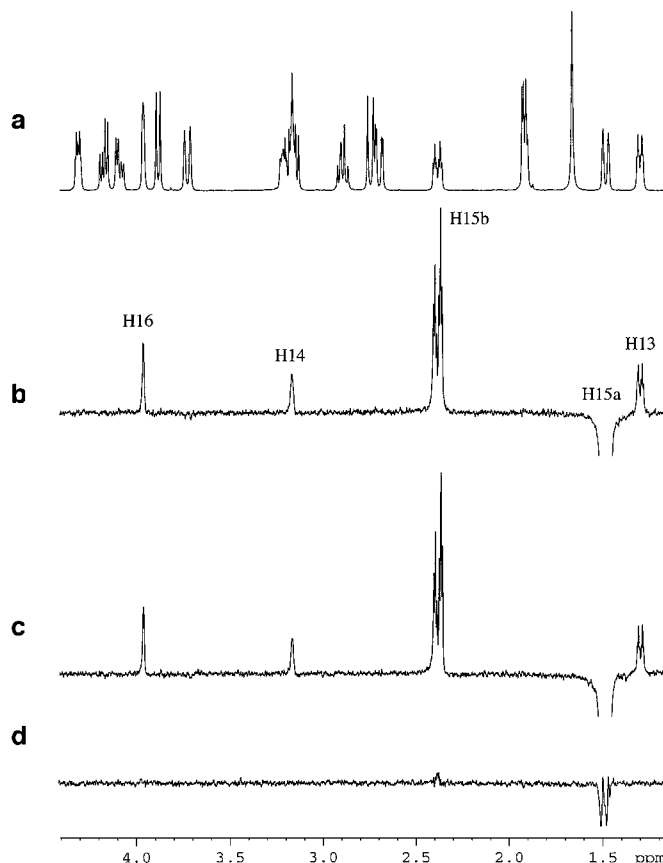


FIG. 5. (a) Conventional 500-MHz 1H spectrum of strychnine in $CDCl_3$; (b–d) 1D GROESY spectra at (b) 298 K, 20 Hz, spinning; (c) room temperature, heater off; (d) 298 K, no spinning. The H15a was selectively inverted by a 20-ms 1% truncated selective pulse. A 3-KHz, CW spin lock of 500 ms was used as a ROE mixing time.

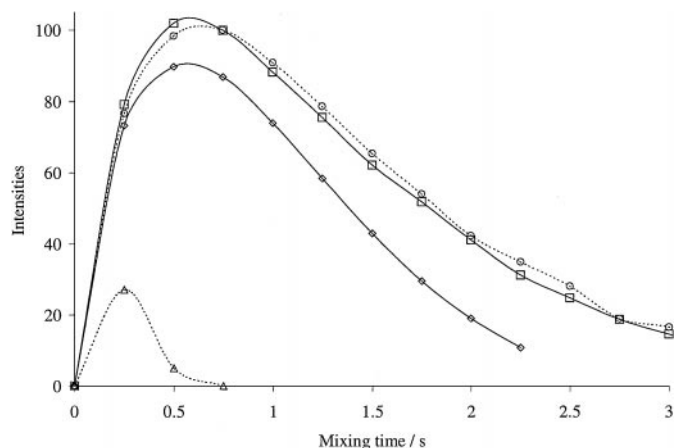


FIG. 6. ROE build-up curves using the GROESY experiment with and without sample spinning. The spin-lock time was varied from 0.25 to 3.25 s and all other experimental parameters are as described in legend of Fig. 5. Four different data are represented: (----○----) rotation at room temperature (296 K); (—□—) rotation at 298 K; (—◇—) no rotation at room temperature (296 K); and (----△----) no rotation at 298 K. All signal intensities have been normalized to the first mentioned data.

maximum NOE attainable in shorter mixing time values (Fig. 6), also depending on gradient strength, and in cases of severe convection effects there is an oscillatory decay of the signal for large mixing times as described earlier for diffusion coefficients. In our hands, the sensitivity of convection-compensated NOE and ROE sequences (18) shown decreased sensitivity probably due to its major dependence on pulse calibrations and general set-up. In addition, doubling the stimulated echo sequence and placing an additional gradient in the center could introduce new artifacts arising from coherence transfer processes in coupled spin systems.

CONCLUSIONS

Temperature gradients in the NMR sample tube can entail important errors in the experimental determination of translational self-diffusion coefficients by NMR. These effects can become critical for samples dissolved in nonviscous solvents such as chloroform, acetone, acetonitrile, or methanol, even at low temperature, and to a minor extent in benzene or toluene. In addition, they can be important enough even in viscous solvents below 300–305 K, i.e., in aqueous or DMSO solutions. For this reason, they must be detected and minimized if rigorous measurements are desired.

We have shown that the simple use of sample spinning on the conventional BPLED pulse schemes affords reliable measurements, comparable to those obtained with a convection-compensated sequence, and also affords improved line resolution. Similar results were also obtained when using other NMR diffusion schemes such as STE or the regular LED sequences. In addition, this simple approach can be extrapolated to any NMR pulse scheme involving coherence selection by PFG, in which

the defocusing and refocusing gradients are found well separated and, therefore, convection effects can considerably affect both sensitivity and performance. The effectiveness of the use of sample spinning has been confirmed by several NMR spectrometers in our laboratories and elsewhere equipped with conventional temperature regulation systems.

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