

High-resolution proton solid-state NMR spectroscopy by phase-modulated Lee–Goldburg experiment

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Abstract

In this Letter, we present a pulse scheme for coherent averaging of spin–spin interactions called phase-modulated Lee–Goldburg (PMLG) which is aimed at achieving high-resolution proton NMR spectra in the solid state. Our objective is mainly to discuss the design and execution of this experiment and to show some preliminary experimental results as evidence of the far-reaching potentials of this technique. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

The appearance of many sophisticated techniques in the field of solid-state NMR in recent years has expedited the scope of its application to analytical investigations of a wide range of solid samples. While CP-MAS and proton decoupling have proven to be effective in achieving high-resolution spectra of nuclei like ^{13}C and ^{31}P [1,2], DOR, DAS and MQMAS together with the above have been able to yield similar spectra in the case of quadrupolar samples like ^{23}Na and ^{87}Rb [3–6]. These methodologies help to overcome the problems inherent in these samples, namely, long relaxation times, low sensitivity, strong heteronuclear dipolar interactions between these spins and the abundant proton spins, chemical shift anisotropy (CSA), and quadrupolar interactions contributing both in the first and second order. De-

spite advancements along these lines, obtaining high-resolution proton spectra in solids nonetheless remains difficult, while possibilities for its practical use keep growing in fields ranging from structural dynamics to biomolecular investigation. The main line-broadening interaction in the case of protons is the strong homonuclear dipolar interactions which usually fall in the range of 30–40 kHz. Being homogeneous in character, unlike heteronuclear dipolar interactions, it can be successfully removed only if the sample is spun at speeds greater than the strength of the interaction. Hence, it is transparent that even at the ‘high’ speeds available today that touch 35 kHz, obtaining a well-resolved proton spectra in most solids remains elusive.

Two approaches that have played an important role in trying to resolve this issue have been the Lee–Goldburg (LG) method [7] and its variant, the frequency-switched LG experiments (FSLG) [8], and homonuclear multiple pulse decoupling [9], starting from the basic WHH-4 cycle [10]. A great deal of attention has been on the latter, resulting in the

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design and implementation of various sophisticated multipulse techniques: e.g., MREV-8 [11], BR-24 [12], TREV-8 [13] and MSHOT-3 [14]. A related advancement has been the successful combination of multiple pulse and MAS to obtain high-resolution CRAMPS proton spectra [13,15]. Recently improved proton spectra were achieved by combining WHH-4 and high-speed MAS [16]. On the other hand, relatively few developments have been made in the direction of exploiting the LG experiment in order to obtain a high-resolution proton spectra. This methodology relies on the fact that, under the application of an off-resonance continuous rf field at $\omega_1/\sqrt{2}$, with ω_1 being the rf field strength, the spins rotate around an effective field tilted away from the static field direction by the magic angle. The zero-order average of the dipolar Hamiltonian vanishes in such a case, paving the way for an enhanced line narrowing otherwise made difficult by the strong homonuclear dipolar interactions. An improved line narrowing is achieved by applying the FSLG experiment, in which the continuous rf irradiation frequency is switched between the two LG conditions $\pm \omega_1/\sqrt{2}$ after each 2π rotation of the proton magnetization about the effective field. A shift in the phase by π is required together with each frequency switch. While Waugh and Mehring accomplished this on static samples by shifting a static magnetic field arranged parallel to the main magnetic field [8], Bielecki et al. did the same on rotating samples by switching the frequency of the irradiation field keeping the static field constant [17,18], thus making good use of the capabilities of the modern frequency synthesizers. Effective use of the FSLG scheme in combination with MAS for homonuclear decoupling has been employed in the measurement of CSA and heteronuclear dipolar couplings [19] and for polarization transfer measurements [20]. The relevance of this methodology has been further appreciated, especially in the field of biological studies [21]. This can be ascribed to the fact that FSLG is easier to implement than multipulse techniques and is less sensitive to both the experimental imperfections and interplay of simultaneous averaging processes, the latter affecting the performance of multipulse techniques critically.

Since the preliminary work carried out by Bielecki et al. [17,18] and Levitt et al. [22] that demonstrated the practical feasibility of FSLG, exploratory

work in this field has resulted in two main advances: (1) the heteronuclear dipolar correlation spectroscopy of solids under high-speed and high-field conditions [23] and (2) the observation of J couplings via correlation spectroscopy in solids [24]. Both of these classes of experiments efficiently use FSLG homonuclear decoupling in the t_1 dimension of a two-dimensional experiment. It may be noted in passing that efficient use of LG during cross-polarization for homonuclear decoupling can enhance the transient oscillations observed during CP-MAS experiments [25].

The realization of FSLG by Bielecki et al. [17,18] and Levitt et al. [22] called for introducing observation windows in the sequence which could have been a major reason for the relatively poor spectra obtained in their experiments. Considering the ease with which the FSLG methodology can be implemented, we have theoretically and numerically searched to improve this technique or to find other variants for an enhanced performance. We realized that a better way to do this was to implement the sequence two-dimensionally. The t_1 projection of such an experiment readily yields a high-resolution spectrum while the t_2 projection carries the normal MAS spectrum. Another advantage of such an experiment is the straightforward extension to correlation investigations which could be beneficial in a variety of scenarios.

With the above in mind, we introduce a new class of FSLG experiments named 'PMLG' (phase-modulated Lee–Goldburg). This new scheme accomplishes line narrowing not by frequency switching as in FSLG but by a series of pulses with well-defined phases. The efficiency of this methodology compared to FSLG may be debated, because FSLG uses only one step in the jumping of frequency, together with a phase shift of π , and PMLG uses a large number of steps given by the number of pulses. This could add errors cumulatively in the experiment, although the necessity for a simultaneous shift in both frequency and phase is avoided. Nevertheless, this point of concern does not look alarming when viewing the experimental spectra, a fact that has been substantiated by numerical simulations. Some exceptions will be pointed out later in Section 3. At the same time, this particular structure of PMLG allows for much versatility to control and manipulate

the amplitudes and phases of the pulses, a situation which, in the normal case, the FSLG experiment may not be able to perform. This in itself opens up many possibilities to improve the efficiency of this methodology towards line narrowing and incorporate such sequences in other related experiments, e.g., heterocorrelation experiments. In this Letter, we discuss some of the salient features of this technique with respect to the experimental protocols and performance levels. It is expected that this approach will further enliven this field offering possibilities for pulse shaping and amplitude and phase modulation that should be able to take care of the higher-order terms in the average Hamiltonian making the quest for a high-resolution NMR of the solid state practically viable.

2. Pulse and phase considerations

As the name suggests, PMLG achieves line narrowing by a sequence consisting of a burst of rf pulses with the phase sequentially varying from one pulse to another. This methodology was sought after as an analogue of FSLG but still being less dependent on hardware instability and as a method that gives an option for pulse shaping and modulation. Furthermore, the experiment successfully avoids the errors that arise in FSLG experiments due to frequency switching in addition to the reversal of phases discussed earlier as this technique requires only a reversal of phases like in multipulse sequences.

In the following, we deal with the theoretical details of the selection of the phase angles. The general form of the rf field Hamiltonian in the normal rotating frame for an LG unit of duration t_{LG} , $(n-1)t_{LG} < t < nt_{LG}$ and $n = 1, 2, \dots$ can be written as

$$\mathcal{H}_{rf} = \omega_1 \left[I_x \cos(\omega_{PMLG}t + \theta_{LG}) + I_y \sin(\omega_{PMLG}t + \theta_{LG}) \right] \quad (1)$$

where ω_1 is the rf field strength, ω_{PMLG} the phase modulation frequency and θ_{LG} an arbitrary phase angle. The operator that transforms the spin Hamiltonian of a set of coupled homonuclear spins $I = 1/2$, $\mathcal{H}_H + \mathcal{H}_{CS} + \mathcal{H}_{rf}$, to the rf interaction frame can be made periodic and the zero order average dipole–di-

pole Hamiltonian, $\overline{\mathcal{H}}_{II}^0$, to vanish [9], considering an LG irradiation unit, for the following parameters:

$$|\omega_{PMLG}| = \sqrt{\frac{1}{2}} \omega_1, \quad t_{LG} = \sqrt{\frac{2}{3}} \frac{2\pi}{\omega_1}. \quad (2)$$

From this it is evident that the angle through which the rf precesses in one unit of LG in the rotating frame is given by

$$\alpha_{LG} = |\omega_{PMLG}| t_{LG} = 207.8^\circ. \quad (3)$$

For $0 < t < t_{LG}$, $\omega_{LG} > 0$ and $\theta_{LG} = 0$ the average chemical shift Hamiltonian in the rf interaction representation has the form

$$\overline{\mathcal{H}}_{CS}^0 = \sum_i \Delta \omega_i \cos \theta_m (I_{zi} \cos \theta_m + I_{xi} \sin \theta_m) \quad (4)$$

where $\Delta \omega_i$ is the chemical shift of spin i , and θ_m the magic angle of 54.7° . The same chemical shift Hamiltonian (Eq. (4)) is obtained when $0 < t < t_{LG}$, $\omega_{PMLG} < 0$ and $\theta_{LG} = \pi$. To maintain this form of $\overline{\mathcal{H}}_{CS}^0$ (Eq. (4)) for $(n-1)t_{LG} < t < nt_{LG}$, the angle $\theta_{LG}^n \neq 0$ must be adjusted accordingly to the accumulated rf phase. Here n represents the number of times an LG unit is repeated. We denote the precession of the rf field for $\omega_{PMLG} > 0$ as x and that for $\omega_{PMLG} < 0$ as \bar{x} . A full unit of PMLG, for symmetrization arguments [8,9], takes the form $x\bar{x}$ or $\bar{x}x$. The trajectory that the rf takes for a single unit of $x\bar{x}$ ($\theta_{LG}(x) = 0$, $\theta_{LG}(\bar{x}) = 207.8^\circ - 180^\circ = 27.8^\circ$) and $\bar{x}x$ ($\theta_{LG}(\bar{x}) = 180^\circ$, $\theta_{LG}(x) = 207.8^\circ$) as it traverses a certain path and comes back to the originating point is given in Fig. 1a,b, respectively. Hence,

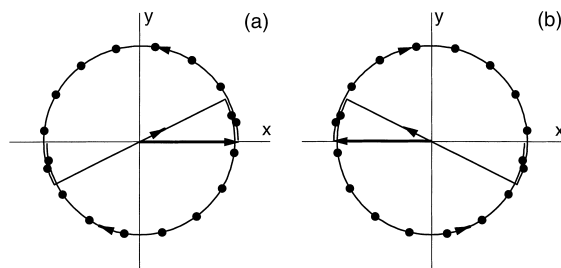


Fig. 1. A schematic showing the trajectory of the rf field during (a) $x\bar{x}$ and (b) $\bar{x}x$ sequences of the PMLG experiment. The positions along the trajectory corresponding to which phases of the pulsed version of the PMLG experiment are chosen are also shown for both $x\bar{x}$ and $\bar{x}x$ experiments. The trajectories are made smaller for visual clarity.

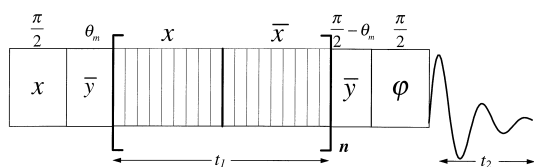


Fig. 2. Pulse sequence used for the FPMPLG experiment.

under one $x\bar{x}$ of the PMLG sequence, the rf field precesses from 0° to 208° during x and then to 180° , after a 180° flip, during \bar{x} . This constitutes a unit of PMLG which is executed by a series of pulses with well-defined phases, α_i , for the i th pulse.

From Eq. (2), for a given rf field strength and number of pulses m , the duration of each pulse in the PMLG unit, the pulse width, is t_{LG}/m . The increment in the phase angle of each pulse amounts to $\Delta\alpha = 207.8/m$ for either x or \bar{x} . Although the number of pulses, that constitute the PMLG units has to be as high as possible for a smooth rf profile, it will result in very short time scales for the pulse width which will culminate in undesirable phase glitches, thereby adversely affecting the performance of the sequence. Hence, a compromise will need to be made between the number of pulses and pulse width. Since the latter is the more stringent criterion from a hardware point of view, it is chosen to be ca. $1\ \mu\text{s}$ for optimum performance.

With the above in mind, the rf field strength was fixed at 82 kHz and the number of pulses m to be 9 which sets the pulse width at $1.1\ \mu\text{s}$ and $\Delta\alpha$ at 23.09° . This requires the use of 18 pulses for either $x\bar{x}$ or $\bar{x}x$. However, an experiment involving both $x\bar{x}$ and $\bar{x}x$, which may help in compensating for the rf pulse and phase imperfections as well as B_1 field inhomogeneity, will dictate the use of 36 pulses each having a distinct phase. While doing such an experiment may be quite necessary, it is often much difficult to work with 36 pulses each having a distinct phase as a limitation imposed by certain commercial spectrometers.

Fig. 1a,b shows the 18 points along the pathway of the rf that determine the phases of the pulses applied for $x\bar{x}$ and $\bar{x}x$, respectively. We start the evolution from an angle of 11.54° and then each successive angle is chosen by addition of $\Delta\alpha$ given by 23.09° in our case. Hence for the $x\bar{x}$ unit, the

phase $\phi_i = 11.54^\circ + (i-1)23.09^\circ$ for pulses $i = 1-9$, and $\phi_i = 16.26^\circ - (i-10)23.09^\circ$ for pulses $i = 10-18$. Similarly, for the $\bar{x}x$ unit, $\phi_i = 168.45^\circ - (i-1)23.09^\circ$ for pulses $i = 1-9$, and $\phi_i = 163.73^\circ + (i-10)23.09^\circ$ for $i = 10-18$. If there is a limitation on the number of phases that can be used simultaneously, 16 pulses with properly chosen phases can be employed instead of the 36 required. The results of such an implementation will also be shown in the following.

3. Experimental

The pulse sequence is highlighted in Fig. 2. Here we use $x\bar{x}$ units with sampling done after three of them, $(x\bar{x})_3$. Each unit of $x\bar{x}$ had a duration of 20 μs . The PMLG sequence in the t_1 dimension follows preparatory pulses that create initial magnetization in

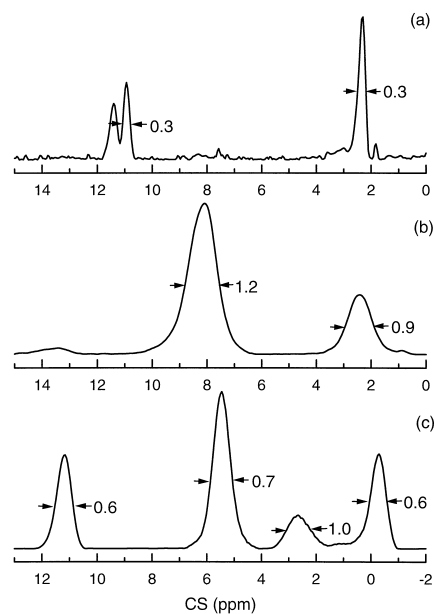


Fig. 3. The t_1 projections from the two-dimensional PMLG experiment performed on (a) malonic acid ($(\text{COOH})_2\text{CH}_2$), (b) glycine ($\text{NH}_2\text{CH}_2\text{COOH}$) and (c) mono-ethyl fumarate ($\text{CH}_3\text{CH}_2\text{COO}(\text{CH}_2)_2\text{COOH}$). The experimental details are given in the text, the spinning speed being same as the corresponding ones in the two-dimensional experiment. The splitting of the carboxyl lines in the case of malonic acid was of the order of 0.4 ppm. The ppm scale was shifted to obtain the CH_2 lines at 2.6 ppm.

the plane perpendicular to the direction of effective chemical shift Hamiltonian in Eq. (4) [23]. This is achieved by two pulses, as depicted in Fig. 2, with the phase of the first pulse incremented as in a TPPI scheme [26]. The PMLG sequence is followed by a $90^\circ - \theta_m = 35.3^\circ$ pulse, which transfers the precession plane to a plane perpendicular to the xy plane. The last 90° pulse along with the receiver is cycled as $x, y, -x, -y$ to leave the z component. The t_1 increment is not rotor synchronized in the experiment and observation of spinning sidebands in the t_1 dimension is possible.

All the experiments were carried out on a Bruker DSX-300 MHz spectrometer using a 4 mm CRAMPS probe. The B_1 field inhomogeneity was of the order of 4% as shown by a nutation experiment. Samples of malonic acid, glycine and mono-ethyl fumarate were investigated to check the efficiency of PMLG. No confinement of the samples was done. It may be

mentioned that all the setup aspects like inspecting the scaling factor were done on adamantane. The scaling factor was adjusted to 0.58 by modifying the rf power looking at the single proton resonance of adamantane.

As stated earlier, a constant rf power of 82 kHz was used in all the experiments. The duration of a 90° pulse was $3.1 \mu\text{s}$ while the magic angle pulse θ_m was $1.9 \mu\text{s}$. Spinning speeds of 15, 13 and 10 kHz were used for glycine, monoethyl fumarate and malonic acid, respectively, for which the relaxation delays were 5, 15 and 25 s on that order. As mentioned in Section 2, another implementation of PMLG with 16 different phases was used for the purpose of demonstration employing an rf power of 85 kHz. In this experiment, phase increment is given by $\Delta\alpha = 360.0/16 = 22.5$. For the $x\bar{x}$ unit, the phase $\phi_i = 11.25^\circ + (i-1)22.5^\circ$ for pulses $i = 1-9$, and $\phi_i = 11.25^\circ - (i-10)22.5^\circ$ for pulses $i = 10-18$. Simi-

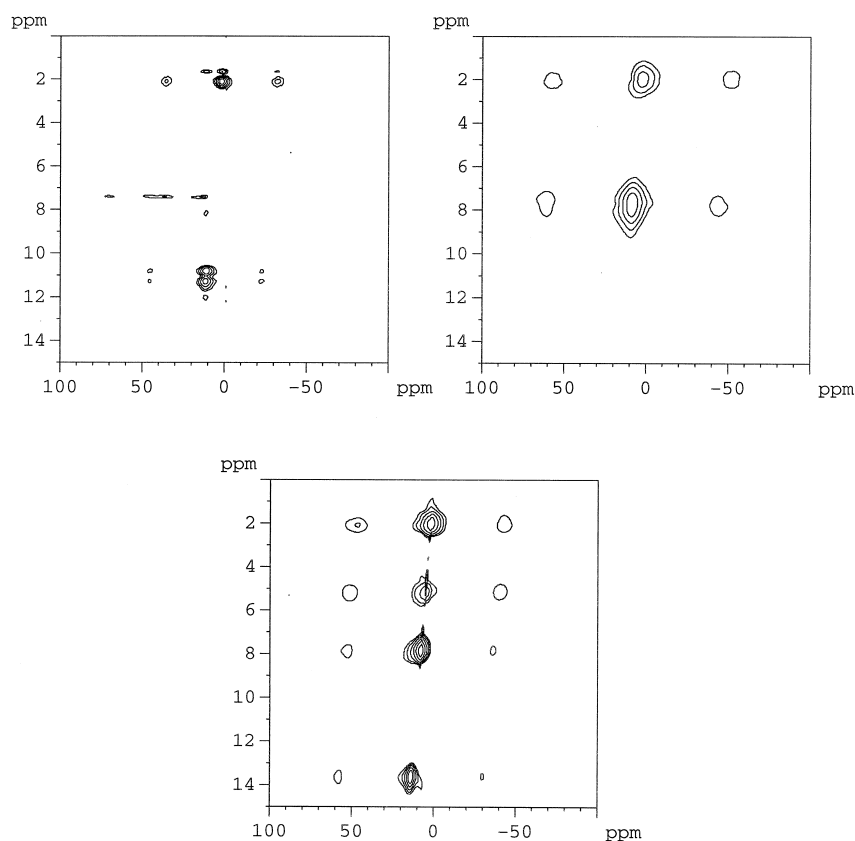


Fig. 4. Contour plots of the two-dimensional PMLG experiment performed on (a) malonic acid, (b) glycine and (c) mono-ethyl fumarate.

larly, for the $\bar{x}x$ unit, $\phi_i = 168.75^\circ - (i - 1)22.5^\circ$ for pulses $i = 1-9$, and $\phi_i = 168.75^\circ + (i - 10)22.5^\circ$ for $i = 10-18$. The pulse length in this case was 1 μ s.

4. Results and discussions

Fig. 3a shows the t_1 projection of the spectrum of malonic acid. The resolution of the carboxyl and methylene resonance together with the splitting in the carboxyl line to about half way down can be appreciated from the figure. The line widths obtained with PMLG are given in the figure. There is a zero peak observed in all experiments which is not shown. This could be because no pulse imperfection compensation phase cycling was employed. Fig. 3b,c show PMLG spectra of glycine and mono-ethyl fumarate, respectively. The observation of a diagonal line in certain spectra, for e.g., mono-ethyl fumarate, may be due to the technical imperfections arising from the phase switching between the pulses. Work is in progress to remedy this deleterious effect. Fig. 4 shows the two-dimensional contour plots of all the three samples mentioned above obtained under the conditions which were used to get the t_1 projections. An essential point worth mentioning is the clear evidence of resolution in the two-dimensional pro-

jection, thus enabling the technique to be used for correlation experiments involving proton–proton or heterocorrelation spectroscopy, both of which can shed light on the structure and dynamics of systems under investigation.

To check the properties of PMLG further, an experiment was carried out on malonic acid with $(\bar{x}\bar{x})_8$ shown in Fig. 5a and $(\bar{x}\bar{x})_4(\bar{x}\bar{x})_4$ shown in Fig. 5b. The spinning speed employed was 13 888 Hz which was synchronized with $(\bar{x}\bar{x})_4$. From the figure it is evident that the decoupling efficiency of $(\bar{x}\bar{x})_8$ has drastically been reduced in accordance with the theory [28]. The $(\bar{x}\bar{x})_4(\bar{x}\bar{x})_4$ pulse scheme improves the spectral resolution significantly. In these experiments, an implementation with 16 distinct phases was used, as described previously, and no attempts were made to obtain phase-sensitive spectrum. Normal CYCLOPS [27] phase cycling was utilized. A noteworthy point to be made is the lack of artifacts of any kind in this case, such as zero peak or quadrature images.

We hope that this preliminary communication will serve as an indication of the tremendous potential of this class of experiment involving phase modulation and will open up new avenues in the study of biological macromolecules and pulse technique developments. A judicious implementation of this experiment has already enabled the study of the

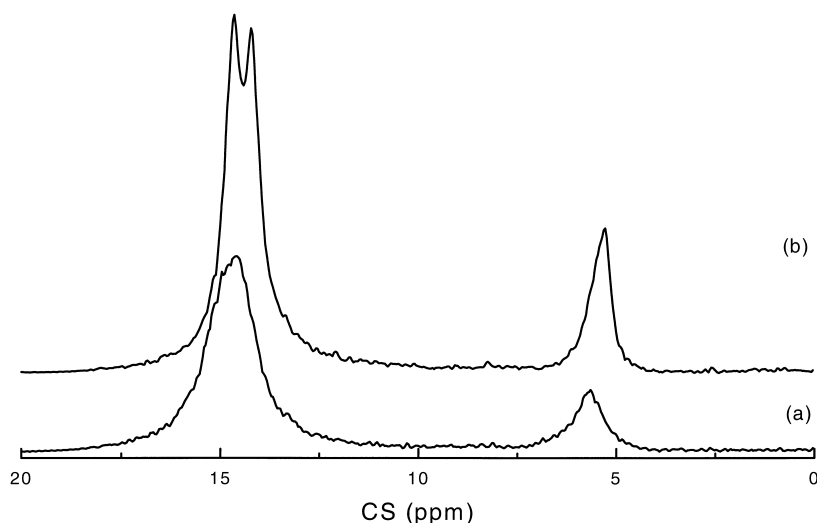


Fig. 5. PMLG spectrum of malonic acid acquired with (a) $(\bar{x}\bar{x})_8$ and (b) $(\bar{x}\bar{x})_4(\bar{x}\bar{x})_4$ sequences with the latter showing clear improvement over the former. The spinning speed used was 13 888 Hz.

dynamics of water molecules adsorbed on CdS nanocrystalline surfaces feasible [29].

5. Conclusions

A new approach of using LG, PMLG, has been outlined here as a coherent averaging process towards obtaining high-resolution proton spectra in the solid state. The possible imperfections that can interfere with the experimental performance are the sense of $\bar{x}\bar{x}$ and $\bar{x}x$ being slightly different, obstructing the *rf* field mismatch compensation and the transition in the phases between the pulses that can affect the signals arising from strong interaction environments. The success of the PMLG experiment demonstrated above within the framework of the well-known limitations is expected to infuse greater interest in the NMR spectroscopic community and manufacturers to make dedicated hardware especially for use in solid-state NMR which will greatly enhance its potential to achieve high-resolution proton NMR spectra of biological macromolecules in solid state in the long run. It is worth re-emphasizing the fact that it is quite simple to execute this experiment in any standard spectrometer. Both FSLG and PMLG suppress only the zero- and first-order terms in the average Hamiltonian. The whole concept of modulation can be further extended encompassing both phase and amplitude, thereby further increasing the efficiency of this experiment. This combination of phase and amplitude modulation may result in suppressing the effects of the higher-order terms in the average Hamiltonian. At the same time, we realize that much more effort needs to be made at the hardware level to make this experiment commonplace. Both numerical and analytical investigations are underway to shed more light on the underlying mechanisms of PMLG and they will be presented in a future publication [28].

6. Note added in proof:

While this Letter was being reviewed, Fung et al. [30] have possibly used an experiment similar to PMLG called 'FSLG2' for homonuclear decoupling

in liquid crystals. They note that the need for such an improvisation stemmed from the fact that, in many commercial spectrometers, the requirement of a duration of 1 μ s or less for frequency switching cannot be easily accomplished.

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References

- [1] C.S. Yannoni, *Acc. Chem. Res.* 15 (1982) 201.
- [2] C.A. Fyfe, *Solid-State NMR for Chemists*, CFC Press, Guelph, ON, 1983.
- [3] A. Samoson, E. Lipmaa, A. Pines, *Mol. Phys.* 65 (1988) 1013.
- [4] K.T. Mueller, B.Q. Sun, G.C. Chingas, J.W. Zwanziger, T. Terao, A. Pines, *J. Magn. Reson.* 86 (1990) 470.
- [5] L. Frydman, J.S. Harwood, *J. Am. Chem. Soc.* 117 (1995) 5367.
- [6] S. Steuernagel, *Solid State NMR* 11 (1998) 197.
- [7] M. Lee, W.I. Goldburg, *Phys. Rev. A* 140 (1965) 1261.
- [8] M. Mehring, J.S. Waugh, *Phys. Rev. B* 5 (1972) 3459.
- [9] U. Haeberlen, *High Resolution NMR in Solids*, Academic Press, New York, 1976.
- [10] J.S. Waugh, L.M. Huber, U. Haeberlen, *Phys. Rev. Lett.* 20 (1986) 180.
- [11] W.K. Rhim, D.D. Elleman, R. W. Vaughan, *J. Chem. Phys.* 58 (1973) 1772.
- [12] D.P. Burum, W.K. Rhim, *J. Chem. Phys.* 71 (1979) 944.
- [13] C.E. Bronnimann, B.L. Hawkins, M. Zhang, G.E. Maciel, *Anal. Chem.* 60 (1988) 1743.
- [14] M. Howhy, P.V. Bower, H.J. Jakobsen, N.C. Nielsen, *Chem. Phys. Lett.* 273 (1997) 297.
- [15] B.C. Gerstein, C. Chor, R.G. Pembleton, R.C. Wilson, *J. Phys. Chem.* 81 (1977) 565.
- [16] S. Hafner, H.W. Spiess, *J. Magn. Reson. A* 121 (1996) 160.
- [17] A. Bielecki, A.C. Kolbert, M.H. Levitt, *Chem. Phys. Lett.* 155 (1989) 341.
- [18] A. Bielecki, A.C. Kolbert, H.S.M. de Groot, R.G. Griffin, M.H. Levitt, *Adv. Magn. Reson.* 14 (1990) 111.
- [19] J.D. Cross, P.R. Costa, R.G. Griffin, *J. Chem. Phys.* 108 (1998) 7286.
- [20] M. Baldus, B.H. Meier, *J. Magn. Reson.* 128 (1997) 172.
- [21] A. Ramamoorthy, F.M. Marassi, M. Zasloff, S.J. Opella, *J. Biomol. NMR* 6 (1995) 329.

- [22] M.H. Levitt, A.C. Kolbert, A. Bielecki, D.J. Ruben, *Solid State NMR* 2 (1993) 151.
- [23] E.J. van Rossum, H. Forster, H.J.M. de Groot, *J. Magn. Reson.* 124 (1997) 516.
- [24] A. Lesage, D. Sakellariou, S. Steuernagel, L. Emsley, *J. Am. Chem. Soc.* 120 (1998) 13194.
- [25] B.-J. van Rossum, C.P. de Groot, H.J.M. de Groot, V. Ladizhansky, S. Vega, submitted.
- [26] D. Marion, K. Wüthrich, *Biochem. Biophys. Res. Commun.* 113.
- [27] D. Hoult, F.E. Richards, *Proc. Roy. Soc. London A* 334 (1975) 311.
- [28] E. Vinogradov, S. Vega, in preparation.
- [29] V. Ladizhansky, G. Hodes, S. Vega, submitted.
- [30] B.M. Fung, K. Ermolaev, Y. Nu, *J. Magn. Reson.* 138 (1999) 28.