

Communication

Heteronuclear dipolar recoupling in liquid crystals and solids by PISEMA-type pulse sequences

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Abstract

A pulse sequence is described for the recoupling of heteronuclear dipolar interactions under MAS. The method is similar to the PISEMA experiment, but employs a well-defined amplitude modulation of one of the two radio-frequency fields. The technique is used for measurements of ¹H–¹³C dipolar couplings in unoriented solid and liquid-crystalline samples.

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1. Introduction

The polarization inversion spin exchange at the magic angle (PISEMA) experiment [1] is a widely used technique for measurements of heteronuclear dipolar couplings in membrane-bound peptides and proteins [2–11]. By combining flip-flop, frequency- and phase-switched Lee-Goldburg (LG) homonuclear decoupling [12,13] with cross-polarization (CP) during the evolution period, highly resolved dipolar spectra can be obtained. The PISEMA sequence typically produces spectra in which the dipolar resolution is up to ten times higher [1,14] than in spectra obtained by the conventional separated local field (SLF) method [15]. Another positive aspect of PISEMA is that it has an exceptionally high dipolar scaling factor [1]. The technique is designed for static (non-spinning) samples and has mainly been used in studies of partially oriented systems and single crystals [1–11]. PISEMA has also been applied to selectively labeled solid powders under stationary [16–19] or quasi-stationary (slow magic-angle spinning (MAS) ~1 kHz) conditions [20,21]. For slowly spinning

powdered samples, a dipolar sideband pattern is obtained in the conventional PISEMA experiment [20]. However, for high chemical-site resolution in powders, spinning speeds of the order of 10 kHz or higher are typically required, which also increases the sensitivity by concentrating the signal to the central band. Unfortunately, the standard PISEMA technique cannot be applied at these spinning frequencies since rapid MAS strongly attenuates the dipolar couplings [22].

In this article, we show that a modified PISEMA sequence can be used to measure heteronuclear dipolar couplings in unoriented systems under sample spinning at frequencies up to ~20 kHz. Since rapid MAS suppresses the dipolar couplings, it is necessary to reintroduce these interactions by suitably designed radio-frequency (RF) pulse schemes. Several techniques for heteronuclear recoupling between abundant (typically ¹H) and rare (typically ¹³C or ¹⁵N) spins have been developed [23–30]. For example, sideband versions of on-resonance and Lee-Goldburg CP were recently analyzed and applied to MAS heteronuclear recoupling in solids [31–38]. Borrowing ideas from this approach, we show that heteronuclear recoupling occurs in PISEMA if a modified sideband CP sequence is used. The new MAS technique is demonstrated on both solid amino acids, and on an unoriented liquid crystal exhibiting motionally-averaged dipolar couplings. We focus in this

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communication on measurements of ^1H - ^{13}C dipolar interactions. The approach is, however, equally applicable to other $^1\text{H}_n$ -S systems where S is a rare spin-1/2 nucleus.

2. Pulse sequence

The general two-dimensional (2D) PISEMA pulse sequence is shown in Fig. 1a and the conventional SEMA multiple-pulse cycle, used for stationary samples, is displayed in Fig. 1b. After standard ^1H - ^{13}C CP, the ^1H magnetization is aligned at the magic angle to the static magnetic field by means of a ^1H 35° pulse. It is subsequently spin-locked by off-resonance flip-flop LG irradiation and matched by a phase-alternated ^{13}C RF field. The heteronuclear dipolar couplings are monitored through the oscillations resulting from coherent polarization transfer between ^1H and ^{13}C spins during t_1 . The Hartmann-Hahn (HH) matching condition must be satisfied for efficient spin exchange, and for the case of stationary samples and off-resonance LG irradiation on ^1H spins it reads

$$\omega_{\text{eff,H}} - \omega_{1,\text{C}} = 0, \quad (1)$$

where $\omega_{\text{eff,H}} = (3/2)^{1/2} \gamma_{\text{H}} B_{1,\text{H}}$ and $\omega_{1,\text{C}} = \gamma_{\text{C}} B_{1,\text{C}}$. For a ^1H - ^{13}C spin pair in an oriented system, one obtains a doublet in the dipolar dimension (see Fig. 1c) with a splitting of $\sin(\vartheta_{\text{m}}) \times 2d$, where d is the ^1H - ^{13}C dipolar

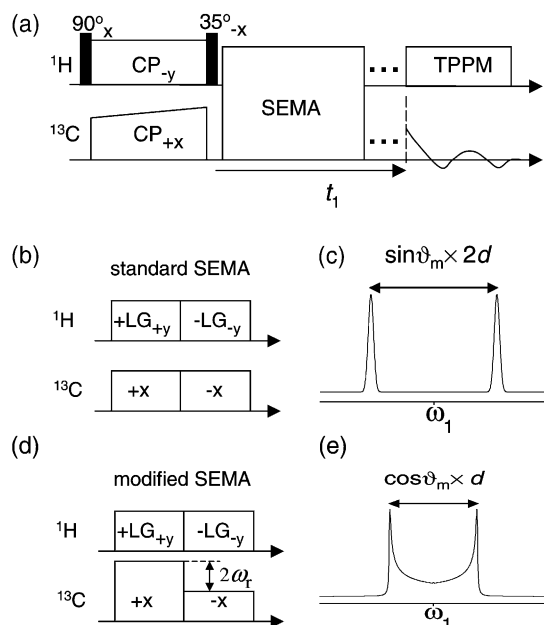


Fig. 1. (a) Pulse sequence for 2D PISEMA spectroscopy. (b) Standard SEMA multiple-pulse cycle. (c) Dipolar spectrum of an oriented system obtained using the standard SEMA sequence on a stationary sample. (d) Modified SEMA multiple-pulse cycle for MAS experiments. (e) Dipolar spectrum of an unoriented system using the modified SEMA sequence under MAS condition.

coupling, $\vartheta_{\text{m}} = 54.7^\circ$ (the magic angle), and $\sin\vartheta_{\text{m}} = 0.816$ is the scaling factor [1].

Under MAS with a spinning frequency ω_{r} , the heteronuclear ^1H - ^{13}C coupling is most efficiently maintained by matching the two RF fields to the first order spinning sidebands [31]

$$\omega_{\text{eff,H}} - \omega_{1,\text{C}} = n\omega_{\text{r}}, \quad n = \pm 1. \quad (2)$$

If continuous wave (CW) RF fields during CP are employed, either the $n = +1$ or -1 sideband can be chosen according to Eq. (2). The theory of off-resonance CW-CP has been worked out in [31,32] and the resulting dipolar powder pattern for a two-spin system is depicted in Fig. 1e. In contrast, the fast periodic inversion of the RF field phase in the PISEMA experiment leads to cancellation of the recoupling effect. Heteronuclear recoupling can, however, be retained by an alternation of the HH matching condition between $n = +1$ and $n = -1$ sidebands synchronized with the phase switching. As shown in Fig. 1d, the SEMA cycle is thus modified by a sideband-alternated HH matching. The theoretical spectral shape of the recoupled dipolar interaction in a powder is the same as with LG-CP (cf. Fig. 1e) [31,32]. The splitting between the two singularities is equal to $\cos(\vartheta_{\text{m}}) \times d$, where $\cos\vartheta_{\text{m}} = 0.577$ is the dipolar scaling factor.

3. Results and discussion

The modified PISEMA sequence discussed above is first demonstrated on solid amino acids. In Fig. 2, we show examples of dipolar cross-sections of $[2\text{-}^{13}\text{C},^{15}\text{N}]$ -L-alanine (Fig. 2a) and $[2\text{-}^{13}\text{C},^{15}\text{N}]$ -glycine (Fig. 2b).

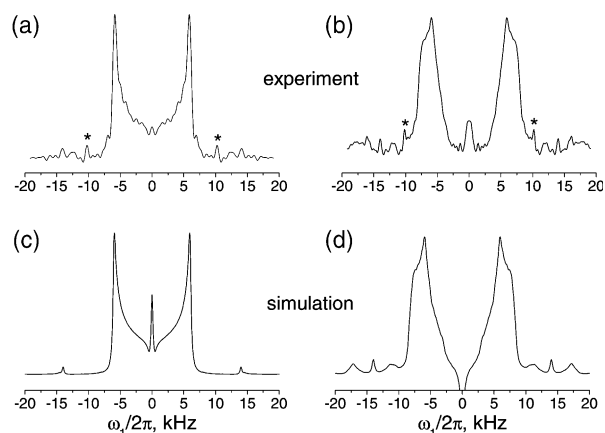


Fig. 2. Experimental dipolar spectra of (a) $[2\text{-}^{13}\text{C},^{15}\text{N}]$ -L-alanine and (b) $[2\text{-}^{13}\text{C},^{15}\text{N}]$ -glycine obtained at a spinning frequency of 14.0 kHz using the modified PISEMA sequence (80 t_1 increments of 26.1 μs). Asterisks indicate sideband aliasing. (c) Numerical simulation for an isolated ^{13}CH group using a ^1H - ^{13}C dipolar coupling of 22.0 kHz. (d) Numerical simulation for an isolated $^{13}\text{CH}_2$ group using a ^1H - ^{13}C dipolar coupling of 21.5 kHz, a ^1H - ^1H interaction of 20.0 kHz, and an H-C-H angle of 109.5° . Both simulations include a ^1H CSA of -2.0 ppm and a ^1H asymmetry parameter of 0. The calculated dipolar powder patterns are rather insensitive to the ^1H CSA parameters.

Both experiments were performed at a spinning frequency of $\omega_r/2\pi = 14.0$ kHz. The spectrum of the ^{13}C spin system in alanine closely resembles the analytical powder pattern calculated for a two-spin system in LG-CP [32] (see Fig. 1e). For the $^{13}\text{CH}_2$ group in glycine, in which the H–C–H bond angle is $\sim 109^\circ$, a more complex heteronuclear-recoupled spectrum is obtained. The best-fit numerical simulations in Fig. 2 reproduce the main features of the experimental line shapes, and the extracted ^1H – ^{13}C dipolar coupling constants (22.0 and 21.5 kHz) agree with results from previous studies of rigid sp^3 -hybridized ^{13}C sites [29,39].

The measurement of motionally averaged dipolar couplings in liquid crystals and biological membranes is of great interest since they provide valuable information on molecular order, structure, and dynamics. We tested the performance of the modified PISEMA pulse sequence for measuring heteronuclear dipolar couplings in an *unoriented* columnar mesophase formed by 1,2,3,5,6,7-hexaethoxy-rufigallol (RufH8O) [40], see Fig. 3a. The molecule was specifically labeled with ^{13}C at the α position of the aliphatic chains. The residual ^1H – ^{13}C dipolar interactions in *oriented* RufH8O were recently estimated by means of various SLF measurements under

stationary conditions [14,39]. It was, for example, found that the α methylene carbon in the R_3 chain (denoted α_3) exhibits a one-bond coupling of 6.9 kHz at 85°C . The experimental dipolar cross-section for this site in powdered RufH8O obtained by the modified PISEMA approach is shown in Fig. 3b ($\omega_r/2\pi = 8.0$ kHz). The line shape is qualitatively different as compared to the one observed for the $^{13}\text{CH}_2$ group in solid glycine (cf. Fig. 2b). The reason for this difference is that in the liquid-crystalline phase, the motionally averaged directions of the two CH bond vectors in the α_3 methylene moiety are collinear. The sharp features in the experimental spectrum in Fig. 3b allow for an accurate determination of the residual ^1H – ^{13}C dipolar coupling d . By reading off the frequency distance $\Delta\omega/2\pi = 5.6$ kHz between the two “horns”, we obtain a coupling of $d = (\Delta\omega/2\pi)/(\sqrt{2} \times \cos\vartheta_m) = 6.9$ kHz which agrees with the value observed in oriented RufH8O [14]. The factor of $\sqrt{2}$ is necessary since the α_3 carbon is coupled to two equivalent protons [41]. The best-fit numerical simulation in Fig. 3c employs a heteronuclear ^1H – ^{13}C dipolar coupling of 7.0 kHz, where the homonuclear ^1H – ^1H interaction and line broadening are taken into account.

The α_3 carbon signal intensity as a function of the dipolar evolution time t_1 for various CP-based pulse sequences is shown in Fig. 4 ($\omega_r/2\pi = 8.0$ kHz). Fig. 4a displays the ^{13}C magnetization build-up curve during sideband-matched CP ($\omega_{1,\text{H}} - \omega_{1,\text{C}} = +\omega_r$) with on-resonance ^1H irradiation. It is clear from Fig. 4b that LG-CP performed under HH sideband conditions ($\omega_{\text{eff,H}} - \omega_{1,\text{C}} = +\omega_r$) results in a longer dipolar oscillation. As shown by others [33], the amplitude of the LG-CP signal can be increased by using polarization inversion (PI) before the evolution period (see Fig. 4c). Flip-flop LG irradiation, as implemented in the modified PISEMA sequence, further prolongs the dipolar oscillation (see Fig. 4d). Finally, Fig. 4e shows the result of a standard PISEMA experiment without alternation of the sideband matching conditions, i.e., the amplitudes of the two RF fields were held constant and matched to the $n = +1$ sideband. As expected, no dipolar recoupling occurs in this case (the small oscillation in the beginning of the signal is due to sample spinning).

While the initial oscillation decay ($t_1 \leq 1$ ms) of the PISEMA time domain signals is due to powder averaging, the damping at longer times can, in theory, be ascribed to $T_{1\rho}$ relaxation and spin diffusion. In practice, however, instrumental artifacts such as RF field inhomogeneity and transients during phase and frequency alternation also contribute to the signal decay [42,43].

Preliminary experiments and numerical simulations indicate that the modified PISEMA technique: (i) more efficiently suppresses the ^1H – ^1H spin diffusion, (ii) is insensitive to ^{13}C resonance offsets and CSA, and (iii) is less sensitive to RF inhomogeneity as compared to

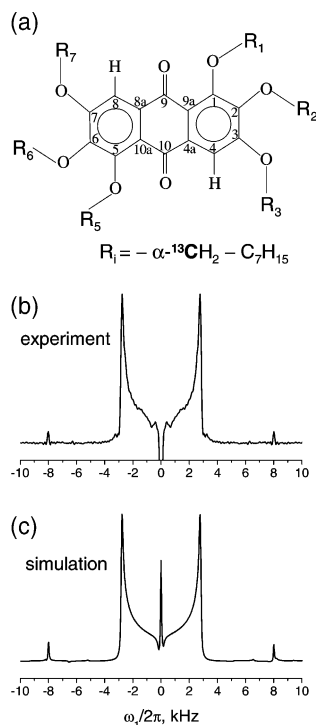


Fig. 3. (a) Chemical structure of 1,2,3,5,6,7-hexaethoxy-rufigallol (RufH8O). (b) Experimental $^{13}\text{C}_\alpha$ dipolar spectrum of unoriented RufH8O in the liquid-crystalline phase at 80°C . The experiment was performed at a spinning frequency of 8.0 kHz using the modified PISEMA sequence (180 t_1 increments of 26.1 μs). (c) Numerical simulation for an isolated $^{13}\text{CH}_2$ group using a ^1H – ^{13}C dipolar coupling of 7.0 kHz, a ^1H – ^1H interaction of 12.0 kHz, and an effective H–C–H angle of 0° .

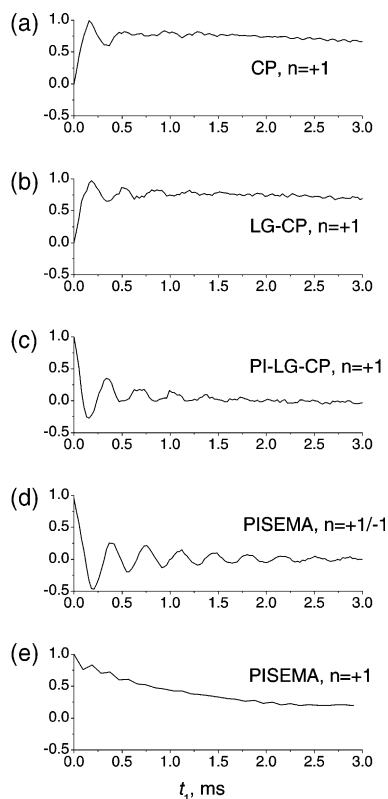


Fig. 4. Experimental $^{13}\text{C}_x$ signal intensity from unoriented Ruh80 versus the contact time for: (a) conventional CP, (b) LG-CP, (c) polarization inversion LG-CP (PI-LG-CP), (d) modified PISEMA, and (e) standard PISEMA. All experimental parameters are identical to the ones given in Fig. 3.

continuous wave LG-CP. Thus, some of the sources of the systematic errors of the estimated dipolar constants are eliminated. The sensitivity to ^1H offsets is, however, as high as in the CW version meaning that the ^1H carrier frequency needs to be carefully adjusted. Notably, proton offsets increase the spectral splittings and hence the apparent coupling constants, as has been discussed for static PISEMA experiments [14,41].

Another potential source of error in CP-based methods is the mismatch of the average B_1 fields in the two frequency channels. (This is also related to the problem of RF inhomogeneity discussed above). In contrast to CW-CP, accurate matching to the $n = +1$ (or $n = -1$) sideband is not critical in the modified PISEMA sequence. Rather, the difference in amplitude between the two alternating ^{13}C RF fields should be matched to twice the spinning frequency (see Fig. 1d). This feature provides additional flexibility in the adjustment of the ^{13}C RF power. In practice at $\omega_r/2\pi = 8.0$ kHz in the meso-phase, we found that it was possible to reduce the ^{13}C B_1 field strength by ~ 15 kHz from the $n = +1/-1$ matching condition without significantly affecting the heteronuclear-recoupled powder pattern. Numerical simulations support this result.

Errors in the proton carrier frequency offset and the difference of the magnitude of the two alternated carbon fields increase the apparent coupling. Therefore, in practice, for an accurate determination of the dipolar couplings, these two parameters are adjusted to provide the *minimum* spectral splitting.

In PISEMA, weak dipolar couplings are truncated in the presence of a strongly coupled spin pair, as has been discussed previously [41]. The effect is merely a distortion in the central part of the spectrum, a small increase of the apparent dipolar splittings, and an additional broadening. An analysis of the influence of multiple weak couplings to remote protons in LG-CP recoupled dipolar spectra, also applicable to PISEMA recoupling, has been carried out for some practical situations [31,32].

The modified PISEMA experiment, in contrast to many other recoupling methods, does not require rotor synchronization. Hence, the RF field amplitudes can be chosen independently of ω_r as long as the flip-flop LG cycle time is short compared to the rotational period $\tau_r = 2\pi/\omega_r$. Numerical simulations indicate that distortions in the recoupled spectral shape occur when the cycle time becomes comparable to the rotor period. This sets the limit on the spinning speed which, for the moderate RF power employed here (see Section 4), is around 16 kHz (corresponding to ca. 2.5 cycles per rotor period). Similarly, lowering the RF power (in order to, e.g., reduce sample heating) would lead to a corresponding decrease of the maximum spinning speed limit.

A potential problem associated with multiple-pulse $^1\text{H}-^1\text{H}$ homonuclear decoupling carried out under rapid MAS is that the decoupling efficiency may degrade due to interference with the sample rotation [44,45]. Fortunately, this problem is not particularly severe for the modified PISEMA technique up to spinning frequencies of ~ 16 kHz using typical ^1H RF power. It is so, because of the short cycle time of the flip-flop LG sequence as has been discussed in the context of CRAMPS experiment [44].

To summarize, we have in this communication introduced a PISEMA-based pulse sequence for measuring $^1\text{H}-^{13}\text{C}$ dipolar couplings under MAS conditions. The method does not require rotor synchronization and has a high dipolar scaling factor. Moreover, the technique efficiently suppresses ^1H spin diffusion, is insensitive to ^{13}C chemical shift parameters, and is rather tolerant to RF inhomogeneity. The modified PISEMA approach was demonstrated on various ^{13}CH and $^{13}\text{CH}_2$ groups in unoriented liquid-crystalline and solid systems. All recoupled powder patterns exhibit sharp features, which allow for an accurate determination of the $^1\text{H}-^{13}\text{C}$ dipolar interaction. In many cases, the heteronuclear couplings can be estimated by direct inspection of the dipolar spectra. Due to its robustness and high scaling factor, we believe that the new recoupling

sequence will become a useful method in studies of partially mobile systems such as unoriented liquid crystals and membranes.

4. Experimental

[α - ^{13}C]-labeled RUFH8O was synthesized according to previously described procedures [46]. A detailed discussion of this compound including its phase diagram can be found elsewhere [39,40]. Isotopically labeled amino acids ([2- ^{13}C , ^{15}N]-L-alanine and [2- ^{13}C , ^{15}N]-glycine) were purchased from Cambridge Isotope Laboratories and used without further purification.

All NMR experiments were performed at a magnetic field of 9.4 T on a Chemagnetics Infinity-400 spectrometer equipped with 6 mm double-resonance and 4 mm triple-resonance MAS probes. Initial ^{13}C transverse magnetization was created by ramped ^1H - ^{13}C CP [47] with nutation frequencies of ~ 60 kHz, and contact times of ~ 1 ms. During the modified SEMA period (see Fig. 1d), the ^1H field strength was 62.5 kHz with a resonance offset of 44.2 kHz. This results in an RF cycle time of 26.1 μs . The ^{13}C field strengths were adjusted so that the difference in amplitude between the two RF fields was equal to $2\omega_r/2\pi$. Heteronuclear decoupling during the detection period in solid and liquid-crystalline phases was achieved by, respectively, 90 and 60 kHz ^1H TPPM irradiation [48].

Numerical simulations were performed using the SIMPSON programming package [49].

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