

COMMUNICATIONS

Two-Dimensional NMR Correlations of Liquid Crystals Using Switched Angle Spinning

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We present the first application of switched angle spinning (SAS) to correlate the first-order dipolar spectrum of a liquid crystalline sample with the isotropic magic angle spinning (MAS) spectrum in a two-dimensional experiment. In this experiment we are able to select the degree of dipolar couplings introduced via mechanical manipulations of the liquid crystal director in a single oriented sample. The ¹⁹F SAS-COSY correlation of iodotrifluoroethylene, an AMX spin system, dissolved in the nematic liquid crystal 4-octylphenyl-2-chloro-4-(4-heptylbenzoyloxy)-benzoate provides assignment of both the *J* and dipolar couplings in a single experiment. This work demonstrates the use of oriented samples and sample spinning to resolve homonuclear dipolar couplings using isotropic chemical shifts. © 2002 Elsevier Science (USA)

Key Words: liquid crystal; switched angle spinning; SAS; dipolar coupling.

INTRODUCTION

Recent developments in the use of dipolar couplings have aided in the determination of structure in oriented samples (1–7). These developments are based on the ability to weakly orient samples in a magnetic field through the use of a solvent environment such as bicelles, phage coat protein, etc. The use of weakly aligned dipolar couplings is expected to greatly improve the accuracy of structure determinations. However, the dipolar couplings measured are typically limited to spins separated by one bond. The utilization of longer range dipolar couplings would allow significantly more structural information to be obtained as demonstrated with deuteration in strongly oriented liquid crystals (8, 9).

Dipolar couplings from more distant spins could be introduced in protein studies by utilizing a more strongly orienting solvent system. However, the observed spectrum would be more complex due to both the increasing strength of the short range

couplings and the addition of the desired long range couplings. The ability to interpret the oriented spectrum depends on finding the appropriate concentration of orienting factors such that the spectrum does not become too complicated by the dipolar couplings or by using novel spectroscopic methods for simplification (10). Complete assignment of the dipolar couplings is then aided by observing the spectrum of the same sample in the absence of the induced orientation.

An alternative to adjusting the extent of dipolar couplings introduced chemically is to utilize spatial averaging by manipulating the liquid crystal alignment director. By first introducing strong alignment chemically, the dipolar couplings can then be scaled by changing the director orientation via mechanical sample spinning (11). The director manipulations allow for the controlled introduction of dipolar couplings and even the ability to scale them to zero by aligning the director at the magic angle. Manipulations of dipolar couplings and other anisotropic interactions in oriented systems have been investigated in order to simplify and assign the information-rich anisotropic spectrum (12–14). One approach involves the use of RF (radiofrequency) multiple-pulse trains to reduce or eliminate the dipolar interaction in a homonuclear dipole coupled spectrum (15). However, the use of spatial averaging has no associated chemical shift scaling factor and is inherently broadband.

Recent studies have utilized director manipulations and analyzed the dependence of the anisotropic interactions in oriented bicelle phases in a series of 1D variable angle experiments (16, 17). Earlier work (18) observed a two-dimensional (2D) correlation of weak dipolar couplings in a liquid crystal introduced with sample rotation at a single axis slightly less than the magic angle without an isotropic dimension. Other investigations utilizing liquid crystal dynamic director experiments have studied the properties of the chemical shift anisotropy (CSA) in a one-dimensional as well as a 2D fashion (19). This work yielded two-dimensional correlations with well resolved cross peaks according to the degree of CSA introduced similar to the earlier work done in solids (20). Assignment of dipolar couplings can become easier for complex samples with the aid of an isotropic

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chemical shift dimension as we demonstrate in liquid crystals and shown previously using switched angle spinning (SAS) in solids (21, 22). Utilizing a homebuilt dynamic angle spinning (DAS) probe, we describe here a 2D experiment with isotropic evolution (at the magic angle) correlated with anisotropic evolution (at an angle different from the magic angle) for a solute dissolved in a nematic liquid crystal.

SPINNING LIQUID CRYSTALS

The feasibility of the type of experiments described here depends on the behavior of the liquid crystalline phase while undergoing sample rotation about a varying axis with respect to the magnetic field. The mechanism for magnetic alignment in nematic liquid crystals is caused by a magnetic force according to the anisotropy of the diamagnetic susceptibility of the molecule ($\Delta\chi = \chi_{\parallel} - \chi_{\perp}$) (23). The director \vec{n} , which describes the orientation axis of the anisotropic motion of the liquid crystal, aligns in a manner that minimizes the magnetic potential energy over the period of one rotor cycle. In liquid crystals with a positive $\Delta\chi$, the director aligns parallel to the spinning axis in the range $0^\circ < \theta_{RL} < 54.7^\circ$, where θ_{RL} is the angle formed with the spinning axis and the magnetic field. As long as θ_{RL} remains in the range of $\theta_{RL} < 54.7^\circ$, the director will remain parallel to the spinning axis. However, spinning at exactly the magic angle for long times (~ 1 s) can cause the sample to disorder due to the lack of any orienting force. Thus above a critical spinning frequency and in an appropriate range of angles, we are able to manipulate the interactions of the sample by changing the orientation of the director. When the director of the liquid crystal is near the magic angle, dipolar, CSA, and first-order quadrupolar interactions are removed due to rapid molecular tumbling about the director, thereby producing an isotropic spectrum in an anisotropic medium.

As an initial demonstration, we have used the liquid crystals as an ordering solvent to allow for the study of solutes dissolved in the liquid crystalline phase. Solute dissolved in nematic liquid crystals have been shown to adopt the ordering properties of the solvent environment in a suitable range of concentrations (23). The ordering abilities of liquid crystals as a solvent allow for the observation of isotropic-anisotropic correlations of dissolved molecules.

INTERACTIONS IN ROTATING LIQUID CRYSTAL SYSTEMS

In the experiment presented here, two averaging processes exist, anisotropic molecular tumbling and sample spinning. Averaging by fast molecular tumbling is the result of time averaging the spatial parts of the dipolar Hamiltonian,

$$H_{Dipolar} = -b_{ij} \left(\frac{3 \cos^2 \theta - 1}{2} \right) [3I_{iz}I_{jz} - \vec{I}_i \cdot \vec{I}_j], \quad [1]$$

where $b_{12} = \mu_0 \gamma_i \gamma_j \hbar / 4\pi r_{ij}^3$, and $\gamma_{i,j}$ are the magnetogyric ratios of the interacting nuclei separated by the internuclear distance r_{ij} at an angle θ with respect to the magnetic field, and \vec{I}_i and \vec{I}_j are the corresponding spin operators for the nuclei. Under anisotropic molecular tumbling and assuming r_{ij} is fixed, the dipolar interaction reduces to

$$\begin{aligned} H_{Dipolar} &= -b_{ij} \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle [3I_{iz}I_{jz} - \vec{I}_i \cdot \vec{I}_j] \\ &= D_{ij}^{aniso} [3I_{iz}I_{jz} - \vec{I}_i \cdot \vec{I}_j] \end{aligned} \quad [2]$$

with D^{aniso} being intrinsic to the liquid crystal phase in a given magnetic field according to the order parameter (24). The dipolar interaction is further averaged by mechanical sample spinning at speeds larger than a critical frequency (13). The dipolar interaction under both averaging processes when the director is aligned with the spinning axis is

$$H_{Dipolar} = D_{ij}^{aniso} \left(\frac{3 \cos^2 \theta_{RL} - 1}{2} \right) [3I_{iz}I_{jz} - \vec{I}_i \cdot \vec{I}_j], \quad [3]$$

where θ_{RL} is the angle of the spinning axis with respect to the magnetic field. Thus the dipolar interaction is completely averaged when the sample is rotated at the magic angle and varying degrees of dipolar coupling can be introduced according to the angle of rotation, $\theta_{RL} < 54.7^\circ$.

In the simple case when the chemical shift difference is larger than the averaged dipolar coupling, $\omega_i - \omega_j \gg D_{ij}$, the truncated interaction is termed first-order dipolar coupling (25) and includes only the $I_z I_z$ spin operators. In the experiments presented in this work, the spectra are first-order with an effective dipolar Hamiltonian of

$$H_{Dipolar} = 2D_{ij}^{aniso} \left(\frac{3 \cos^2 \theta_{RL} - 1}{2} \right) I_{iz}I_{jz}. \quad [4]$$

The splittings observed resulting from the first order dipolar couplings in liquid crystals are the sum of the dipolar and both the isotropic and anisotropic J interactions, explicitly $2D_{ij}^{aniso} + J_{ij}^{aniso} + J_{ij}^{iso}$ (24). Since the anisotropic J and dipolar coupling are essentially indistinguishable and J^{aniso} is typically small in light nuclei, we assume that the anisotropic interaction is dominated by dipolar coupling and include only J^{iso} in the scalar coupling.

The CSA is also averaged to a single orientation value. Although typically small in ^1H , the CSA of ^{19}F nuclei are generally large and the effects of this interaction can be substantial. Again, starting with the static interaction, the CSA is given by

$$\begin{aligned} H_{CSA} &= -\gamma(1 - \langle \sigma_{11} \sin^2 \theta \cos^2 \phi + \sigma_{22} \sin^2 \theta \sin^2 \phi \\ &\quad + \sigma_{33} \cos^2 \theta \rangle) I_{kz}, \end{aligned} \quad [5]$$

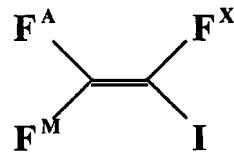
where σ_{ii} are the principal values of the chemical shielding tensor and the angles θ and ϕ describe the orientation of the magnetic field in the principal axis of the chemical shielding tensor. Upon molecular tumbling, the values of θ and ϕ are reduced to their time averaged values thus giving rise to a frequency shift. The net result of sample spinning at the magic angle will provide a spectrum that can be described by the isotropic chemical shift and J couplings. However, spinning at an angle smaller than the magic angle (for $\Delta\chi > 0$) will result in a spectrum including averaged dipolar couplings and chemical shift anisotropies, as well as J couplings. The correlation of the two spinning angles provides limited spectral information about the CSA, and also allows the assignment of the dipolar couplings in a single two-dimensional experiment.

EXPERIMENTAL

Experiments correlating the anisotropic dipolar coupled dimension with the isotropic MAS dimension were performed using a homebuilt DAS probe that allows for angle hopping from zero to the magic angle within 20 ms. This DAS probe used was a modified Chemagnetics (now Varian Inc., Palo Alto, CA) 5-mm HX MAS probe. Angle switching was done by an API Motion (now Danaher Motion, Washington, DC) feedback stepping motor and a pulse programmer triggered motor controller. In addition to rapid movement between angles, a split solenoid coil design was utilized in the probe, which allows for a consistent circuit amplitude and phase independent of the angle of rotation. This is an important property when attempting phase cycling in 2D experiments. Further details of the probe design will be described elsewhere.

Spinning liquid crystal samples commonly requires the use of rotor inserts to prevent sample leaking and degradation. The use of rotor inserts typically reduces the filling factor and may cause spinning instabilities that disrupt the liquid crystalline phase. These problems were circumvented by using Teflon spacers and rubber stoppers. Using appropriately sized rubber stoppers and spacers, stable spinning speeds of >12 kHz for one week with no sample loss for a typical liquid crystal sample were obtained. In a 5-mm outer diameter Chemagnetics pencil rotor, a 3-mm-thick Teflon spacer followed by a 1-mm-thick rubber stopper, then another 5-mm-thick Teflon spacer were inserted on each end of the sample.

The liquid crystal sample of $\sim 25\%$ w/w iodotrifluoroethylene (Oakwood Products, West Columbia, SC) in 4-octylphenyl-2-chloro-4-(4-heptylbenzoyloxy)-benzoate (Acros Organics, Belgium) was prepared under nitrogen. The ^{19}F spectra of iodotrifluoroethylene in all cases were recorded with a center frequency of 376.095042 MHz on a system where TMS resonates at 399.741790 MHz. The ^{19}F spins of iodotrifluoroethylene are labeled A, M, and X corresponding to their respective resonance frequencies.



In order to observe the isotropic-anisotropic correlations a SAS version of the COSY (SAS-COSY) experiment was used as shown in Fig. 3 in the next section. The pulse sequence begins with a 90° pulse, a t_1 evolution period; then a 45° pulse stores part of the signal to allow angle switching to the magic angle after which another 45° pulse recalls the signal for observation in t_2 under magic angle spinning. A 45° pulse was used in order to observe more intense signal in the connected transition cross peaks versus the nonconnected transition cross peaks (26). The SAS-COSY pulse sequence is well suited to the requirement of storing magnetization during the angle switching.

RESULTS

The 1D Characterization

The isotropic spectrum of the neat liquid is shown in Fig. 1A. To demonstrate the ability of MAS to produce an isotropic spectrum, the liquid crystal MAS spectrum is overlaid with the neat liquid isotropic spectrum (Fig. 1B). The relative shifts and splittings due to J coupling are nearly identical in the two spectra. However, there exists an upfield shift of approximately 1 kHz when the $\text{C}_2\text{F}_3\text{I}$ is dissolved in the liquid crystal. The origin of the upfield shift has generally been seen in these types of systems and is most likely a result of “ π stacking” in this highly ordered system (27). Another minor difference in the MAS spectrum in Fig. 1B is the appearance of small spinning sidebands; these are the result of a small amount of thermal disorder that gives rise to a degree of powder-like orientations in the sample and thus spinning sidebands. Figure 1C shows the ^{19}F spectrum when spinning at an angle of 25° from the magnetic field; note the introduction of dipolar splittings while maintaining a first-order type spectrum. In addition, there is a further nonuniform peak position shift from Figs. 1B to 1C due to the CSA.

The spinning angle, θ_{RL} , dependence of the chemical shift was investigated as shown in Fig. 2A. This dependence results from the anisotropic contributions from the chemical shift. The slope of the linear correlation in Fig. 2A contains information about the chemical shift anisotropy as it exists in this anisotropic environment by

$$\delta^{obs} = \delta^{iso} + \delta^{aniso} \cdot P_2(\cos \theta_{RL}), \quad [6]$$

where δ^{iso} is the isotropic chemical shift, δ^{aniso} is the anisotropic component of the CSA as it exists after the motional averaging from the liquid crystal environment, and $P_2(\cos \theta_{RL})$ is the second-order Legendre polynomial. The linear correlation parameters for the three spins are shown in Table 1. Estimates of the CSA can be derived from the δ^{aniso} parameter; however,

interpretation of the CSA tensor requires knowledge of the molecular order parameter which is beyond the scope of this paper.

Shown in Fig. 2B is the spinning angle dependence of the observed dipolar couplings $2D_{ij}^{aniso}(\theta_{RL}) + J$ as it fits to the linear relation

$$\Delta_{ij}^{obs} = 2D_{ij}^{aniso} \cdot P_2(\cos \theta_{RL}) + J_{ij}^{iso}, \quad [7]$$

where Δ_{ij}^{obs} is the observed splitting on both spins i and j and D_{ij}^{aniso} is the dipolar coupling in the liquid crystal. In Fig. 2B we obtain the expected linear dependence of $2D_{ij}^{aniso}(\theta_{RL}) + J_{ij}$ versus $P_2(\cos \theta_{RL})$ with the intercept at the J coupling values, and the sign of J can be derived from the observation of whether the $2D_{ij}^{aniso}(\theta_{RL}) + J$ value passes through zero in combination with the assumed negative sign of the dipolar interaction. In order to determine the absolute sign of the dipolar interaction and thus the sign of the J couplings, it is necessary to know the orientational order parameter of C_2F_3I in the liquid crystal. Based on

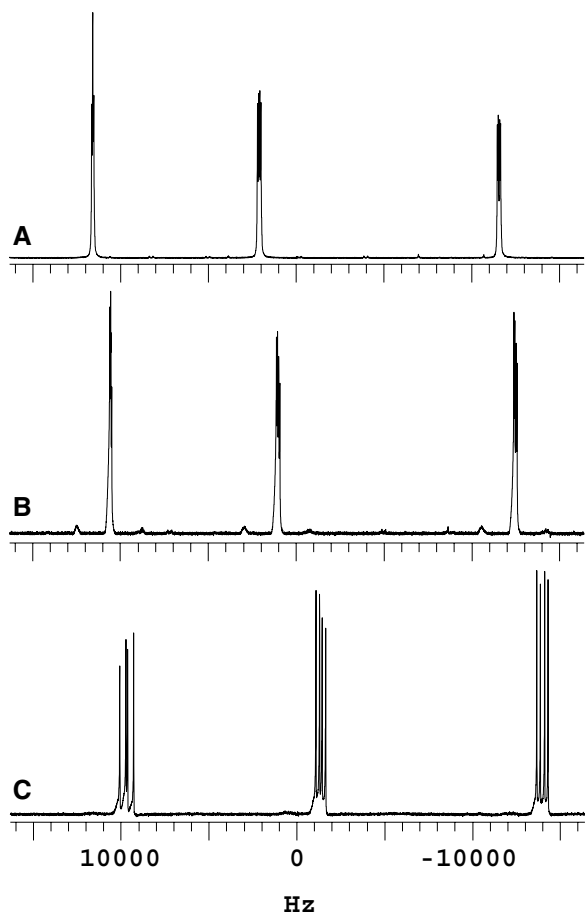


FIG. 1. (A) ^{19}F Spectrum of the neat liquid C_2F_3I . (B) ^{19}F MAS (2 kHz) spectrum of $\sim 25\%$ w/w C_2F_3I in the nematic liquid crystal 4-octylphenyl-2-chloro-4-(4-heptylbenzoyloxy)-benzoate. Note the 1-kHz shift of the neat versus the liquid crystal MAS spectrum. (C) Same as (B) but spinning at 25° with respect to the magnetic field thereby introducing anisotropic interactions.

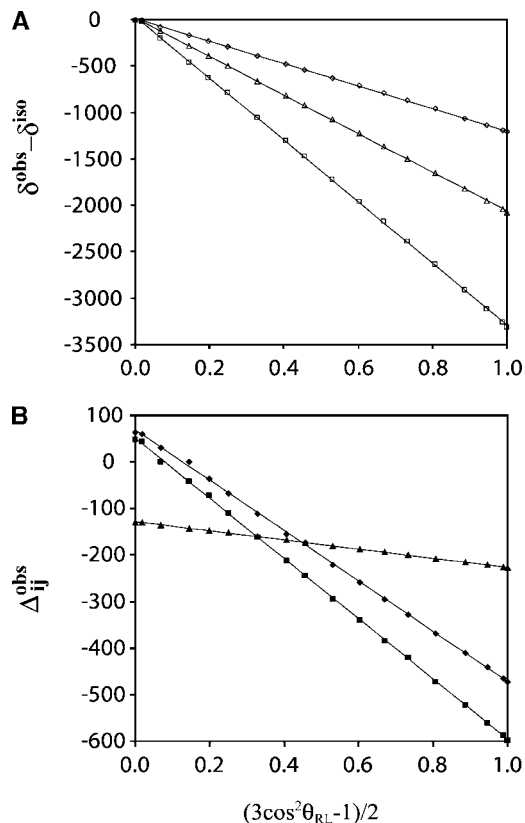


FIG. 2. Spinning axis (θ_{RL}) angular dependence of the observed shift, $\delta_{obs} = \delta_{iso} + \delta_{aniso} \cdot P_2(\cos \theta_{RL})$ (A) and splittings, $\Delta_{ij}^{obs} = 2D_{ij}^{aniso} \cdot P_2(\cos \theta_{RL}) + J_{ij}^{iso}$ (B) for C_2F_3I in the nematic liquid crystal 4-octylphenyl-2-chloro-4-(4-heptylbenzoyloxy)-benzoate. The fitted line parameters for plots A and B are shown in Tables 1 and 2, respectively. In (A) the shift trends are $\diamond = F_A$, $\square = F_M$, and $\triangle = F_X$. In (B) the dipolar splitting trends are $\blacklozenge = \Delta_{AM}$, $\blacksquare = \Delta_{AX}$, and $\blacktriangle = \Delta_{MX}$.

the assumption that the planar molecule would orient with the molecular plane parallel to the liquid crystal director, the dipolar coupling can be assumed to be negative. Courtieu *et al.* (13) have used similar arguments in assigning the sign of the dipolar couplings in C_2F_3Br . We therefore assign J_{AM} and J_{AX} positive values and J_{MX} a negative value since only $2D_{MX}^{aniso}(\theta_{RL}) + J_{MX}$ did not pass through zero (Table 2). Additionally from Fig. 2B we obtain what we would expect to be the $2D_{ij}^{aniso}(0^\circ) + J$ values for a nonspinning sample, where the scaling of the dipolar interaction is unity. The spectra obtained with and without spinning at 0° were nearly identical.

TABLE 1
Chemical Shifts from the Line Fits in Fig. 2A

Spins	δ^{iso} (Hz)	δ_{aniso} (Hz)
A	10578	-1210
M	1061	-3319
X	-12437	-2079

TABLE 2
Coupling Constants from the Line Fits in Fig. 2B

Spins	J^{iso} (Hz)	$2D^{aniso}$ (Hz)
A–M	69.0	–472.4
A–X	51.2	–596.1
M–X	–128.3	–226.4

While spinning at the magic angle, significant thermal disordering occurred within 5 seconds of setting the spinning axis to exactly the magic angle. This thermal disordering was overcome by simply setting the spinning angle slightly less (0.1°) than the magic angle such that slight orienting forces still existed and yet an isotropic spectrum was still observed.

The 2D Correlations

After sufficiently characterizing the interactions in our liquid crystal sample, we turn to the main goal of this work, a demonstration of the correlation of isotropic chemical shifts with anisotropic dipolar couplings using SAS. In order to illustrate the effects of the liquid crystal and SAS on the COSY experiment, we first performed the conventional COSY-45 experiment without angle switching on the neat liquid C_2F_3I . The conventional COSY-45 pulse sequence is simply $90^\circ-t_1-45^\circ-t_2$. Figure 4A illustrates how the J couplings provide cross-peak patterns that indicate the relative signs of the directly connected transitions. The cross-peak regions are enlarged tenfold relative to the scale of the underlying spectrum. The dashed lines act as a guide to reveal the connected cross peaks. The projected axes show that only the J coupling is observable in both dimensions, equivalent to the spectrum in Fig. 1A.

In contrast, the dipolar-isotropic correlation obtained by applying the SAS-COSY experiment of Fig. 3 on the liquid crystal sample is shown in Fig. 4B. The liquid crystalline sample was spinning at 2 kHz and hopped from 25° relative to B_0 in ω_1 to the magic angle in ω_2 . The projected dipolar axis (ω_1 , horizontal) reproduces the spectrum observed by simply spinning at 25° without angle switching. The projected ω_1 dimension appears somewhat broadened due to a combination of a limited number of points sampled during t_1 (512 points) and minor angle

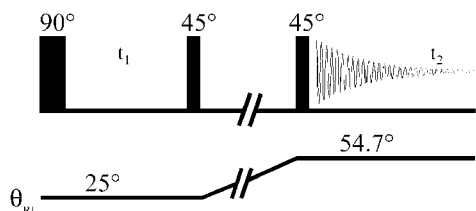


FIG. 3. SAS-COSY pulse sequence used to obtain the spectrum in Fig. 4B. θ_{RL} is the spinning angle with respect to the magnetic field which changes from 25° to the magic angle after the signal is stored with a 45° pulse. The angle switching time was 20 ms.

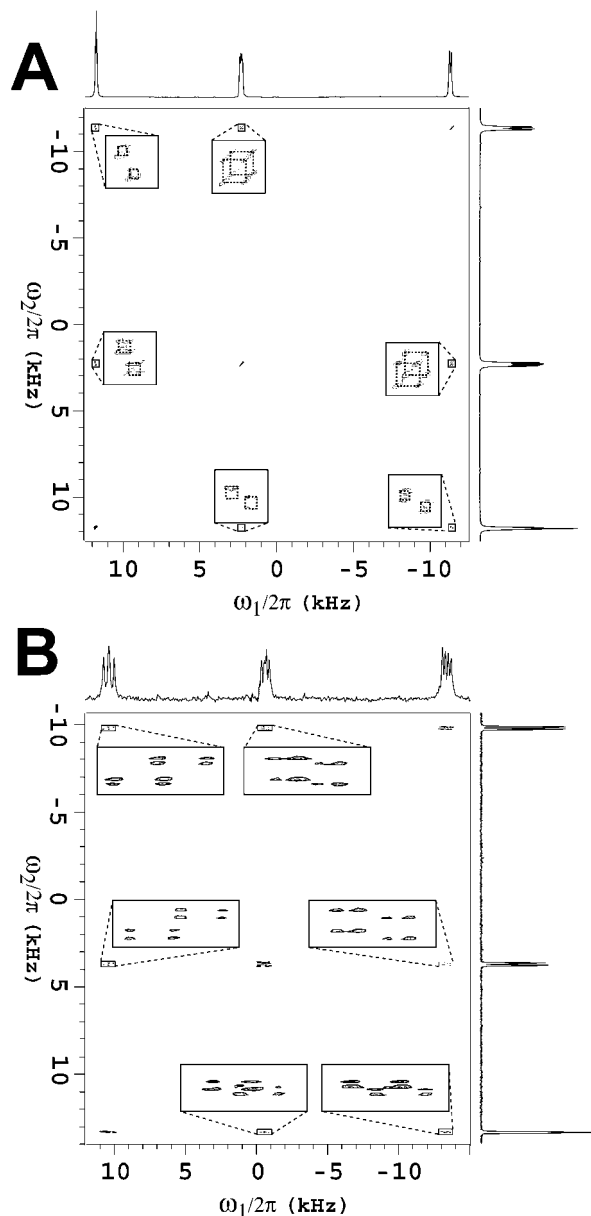


FIG. 4. (A) ^{19}F conventional COSY-45 spectrum of the neat liquid C_2F_3I . (B) ^{19}F isotropic–anisotropic correlation of C_2F_3I in the nematic liquid crystal 4-octylphenyl-2-chloro-4-(4-heptylbenzoyloxy)-benzoate obtained using the SAS-COSY experiment described in the text and shown in Fig. 3 while spinning at 2 kHz. ω_1 is the isotropic dimension (δ, J) and ω_2 is the anisotropic dimension (δ, J, D). The spectra are shown in magnitude mode.

inaccuracies ($<0.1^\circ$). The projection onto isotropic ω_1 or ω_2 dimensions in Fig. 4A is effectively identical to the projection onto the isotropic MAS dimension (ω_2) in Fig. 4B minus solvent effects. The differences in the two projected axes in Fig. 4B reveal not only the introduction of dipolar coupling but also changes in peak positions due to the CSA contributions. The $10\times$ magnified insets show how the $2D^{aniso} + J$ values that evolved during ω_1 are readily revealed by the separation provided by the

J coupling in ω_2 . The measured splittings from the expanded insets were $\Delta_{AM} = 320 \pm 8$ Hz, $\Delta_{MX} = 199 \pm 4$ Hz, and $\Delta_{AX} = 410 \pm 10$ Hz. The values measured in the SAS-COSY experiment agree well with the values as predicted from the linear correlations from Fig. 2.

The cross-peak shapes in this magnitude spectrum are easily understood when considering the spin states of all three fluorines; the directly connected transitions form the corners of the two rectangles in each cross peak, and the slope of the line connecting the center of the two rectangles is determined by the sign of the indirect spin J coupling relative to the observed dipolar coupling (which are all assumed negative in this case). For example, the AX cross peak (upper left) in Fig. 4B has a negative dipolar coupling in ω_1 and a negative J_{MX} in ω_2 , thus the slope of the line connecting the two rectangles is positive. The coupling patterns were reproduced using the Gamma library (28) with the J and $2D^{aniso} + J$ values from Table 2. In the simple system investigated here, the dipolar coupling assignments are trivial; however, this demonstration reveals how the dipolar couplings of only connected transitions are spread out in the second dimension by their respective isotropic chemical shifts.

We expect spin diffusion to be a significant problem when dealing with strongly coupled systems where the barriers to spin diffusion are very low. In order to deal with the potential problems of spin diffusion, we are keeping the couplings weak by spinning at angles close to the magic angle, while minimizing the hopping time where significant dipolar couplings take place.

This initial work was done utilizing the absence of any fluorine in the liquid crystal itself to avoid problems with background signal. However, extending this method to the more general case with protons requires contending with the potentially large resolved signal due to the liquid crystal background. Investigations addressing these issues are currently underway.

CONCLUSIONS

The liquid crystal SAS-COSY experiment described in this paper illustrates how the isotropic chemical shift correlated with dipolar couplings reveals the dipolar coupling network. The ability to manipulate the liquid crystal director and thus the underlying interactions provides a large degree of experimental utility, particularly in a 2D mode. Numerous advantages are gained by performing measurements of dipolar couplings in the SAS mode presented here. First, isotropic chemical shifts are maintained in a 2D correlation without the scaling normally associated with multipulse homonuclear decoupling techniques. In addition, the homonuclear couplings can be scaled at will in order to maintain a first-order type spectrum through the adjustment of the hopping angles. Last, by correlating the dipolar information with the isotropic chemical shifts in one experiment, this type of technique may be useful for more complex correlations involving multiple heteronuclei, and even more information may be extracted for interesting systems including organic systems, proteins, nucleotides, and lipids.

The applicability of this technique is only limited by the selection of a strongly orienting system to allow for the observation of the information rich anisotropic interactions such as dipolar couplings and CSAs. There currently exist a variety of different orienting systems that immediately lend themselves to investigation with the aid of the SAS-COSY technique, for example, organic liquid crystals, phage, bicelles, and polyacrylamide gels. Although in this investigation sample spinning was used to manipulate the director, some orienting systems such as polyacrylamide gels do not require sample spinning. By facilitating the study of residual dipolar couplings in the liquid state, this approach should allow insight into the detailed structure of partially aligned proteins and nucleic acids.

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