

# Variable angle spinning (VAS) experiments for strongly oriented systems: methods development and preliminary results

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**Variable angle spinning (VAS) experiments provide a useful method for measuring long-range dipolar couplings and obtaining isotropic–anisotropic correlation spectra. These experiments make it possible to obtain correlations between isotropic and anisotropic spectra without altering the chemical composition of the sample. They also allow working with very strongly oriented systems that are not accessible to solution-state techniques. In this communication, we discuss recent hardware developments in our laboratory and show representative data from small molecules in strongly oriented liquid-crystalline samples. Copyright © 2008 John Wiley & Sons, Ltd.**

**Keywords:** variable angle spinning (VAS); liquid crystal NMR spectroscopy; oriented samples; residual dipolar couplings

## Introduction

Over the past two decades, solid-state NMR (SSNMR) has emerged as a powerful tool for molecular structure determination. Unlike solution-state NMR where rapid molecular tumbling gives rise to narrow peaks, molecules in the solid state normally retain anisotropic interactions that give rise to broad spectra. On the other hand, anisotropic interactions are a valuable source of structural information. In standard multidimensional SSNMR methodologies, a high-resolution spectrum is obtained in the direct dimension where magic angle sample spinning<sup>[1]</sup> mechanically averages out the anisotropic interactions, and the structural information is recovered in the indirect dimension by the application of rotor synchronized RF pulses.<sup>[2–6]</sup> In variable angle spinning (VAS) experiments, scaling of the dipolar couplings is achieved by changing the direction of the spinning axis relative to  $B_0$ . This method is particularly applicable to samples in the gray area between rigid solids and isotropic solutions. This type of system has previously been investigated using both MAS with sophisticated RF-based dipolar recoupling schemes, e.g. Ref. [7] and with VAS, e.g. Ref. [8]. There is a persistent interest to resolve dipolar couplings in strongly aligned molecules, both because it is possible to measure long-range couplings in these systems<sup>[9]</sup> and because the strongly oriented systems themselves have interesting physical and chemical properties.<sup>[10]</sup> Measurement of dipolar couplings in strongly oriented systems could in principle enable more accurate long-range structural information than the NOE method that is normally used in the conventional solution-state NMR,<sup>[11]</sup> and could provide valuable insight into the behavior of systems such as organic molecules interacting with thermotropic liquid crystals or proteins aligned in a membrane. However, strongly aligned molecules often have higher-order couplings that are too complex to resolve in a one-dimensional spectrum. VAS methods have the potential to combine the best features of solution- and solid-state methods in one experiment without altering the sample conditions, such as by changing the temperature or the liquid crystalline concentration.

This is important in thermotropic liquid crystals, where solvent-dependent artifacts are commonly observed, and in membrane protein systems, where the sample of interest may not even be soluble in isotropic solution.

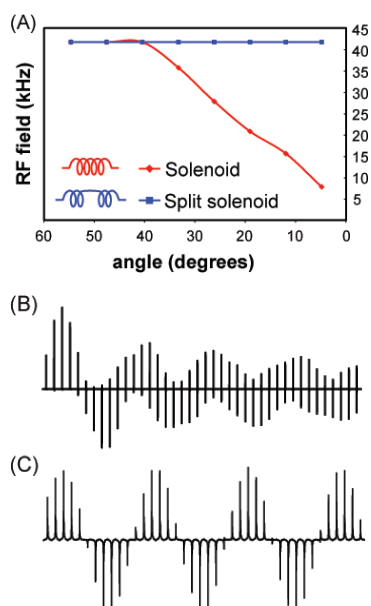
In a related area, there has been extensive progress since the groundbreaking work of Tjandra and Bax to measure residual dipolar couplings<sup>[12]</sup> in molecules dissolved in dilute lyotropic liquid crystals. This method makes it possible to obtain additional structural constraints while retaining the simplicity and high resolution that are characteristic of a solution-state spectrum. However, because long-range interactions are typically not measured, residual dipolar couplings are mostly used as a refinement tool<sup>[13]</sup> rather than as an independent structure determination method. There is a trade-off between obtaining sufficient dipolar couplings and retaining manageable spectral simplicity, but the variable angle method has the potential to resolve this dilemma. When the sample is spinning off the magic angle, there are large couplings and complex, information-rich spectra. Near the magic angle, the couplings are averaged away, leaving more interpretable spectra. If we correlate these two dimensions, either by variable angle correlation spectroscopy (VACS)<sup>[14]</sup> or switched angle spinning (SAS),<sup>[15,16]</sup> it is possible to separate the contribution of dipolar coupling from J coupling, without chemically altering the sample by changing the alignment media between experiments.

Recently, we have developed a new VAS probe that will enable us to carry out VAS experiments on strongly ordered systems.<sup>[17]</sup> This probe addresses many of the challenges that we have previously encountered when performing VAS experiments on complicated

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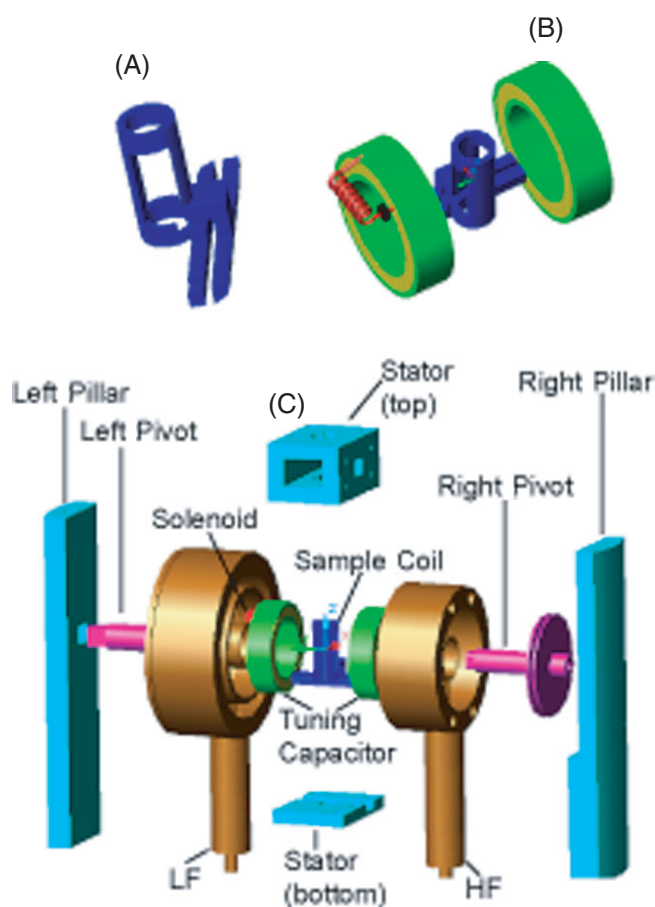


**Figure 1.** Experimental parameters for RF coils used in VAS experiments. (A) Traditional solenoid coils cannot be used for low-angle VAS experiments. Because the direction of the field is along the axis of the coil, it falls off as the spinning axis approaches parallel with the static field. A split solenoid coil can be used to provide a constant RF field at all angles. This approach has been successfully used in previous experiments involving simple sequences using only  $90^\circ$  pulses and continuous wave decoupling. However, the RF homogeneity of this coil is very poor, as illustrated by the proton nutation curve in (B) (shown at 40 kHz field strength). In order to be able to use more complicated sequences, such as the HETCOR with homonuclear decoupling employed below, we have developed the contactless resonator. This coil has very high RF homogeneity, as shown in (C) at the expense of reduced field strength. This trade-off is acceptable because we are working with systems that have attenuated dipolar couplings due to molecular mobility.

organic or biomolecular systems. It includes a coil that moves with the sample in order to maximize the filling factor while keeping the tuning stable. We chose to use a modified Alderman-Grant resonator<sup>[18]</sup> in order to provide good RF homogeneity while minimizing heating due to dielectric loss in conductive biological samples. Unlike a traditional solenoid, this coil design also produces a field that is perpendicular to the main magnetic field, which is necessary to keep the  $B_1$  field constant while changing the spinning angle. Figure 1 illustrates some of the parameters that must be considered when designing a coil for VAS experiments. In order to maintain a good RF connection between the coil and the rest of the probe during hopping and to minimize orientation-dependent tuning changes, we use capacitive coupling between the double-tuned resonator and the rest of the circuit. This hardware will enable us to carry out a series of experiments on strongly oriented molecules in both thermotropic and lyotropic liquid crystals that will be a major focus of our research program. In this article, we demonstrate the utility of this hardware in variable angle experiments on small model systems and discuss future directions.

## Experimental

The VAS experiments presented here were performed using our new contactless resonator. A mechanical drawing of the resonator and related components is shown in Fig. 2. The sample coil itself



**Figure 2.** A detailed view of the contactless resonator and associated components. (A) The sample coil is based on the Alderman-Grant design. It is 4 mm in diameter and has a 4-mm long homogeneous region. (B) The coil is connected to the stationary parts of the probe via coaxial capacitors with copper conductors and teflon dielectric. This structure acts as a double-tuned probe circuit. When the coil reorients, the inner copper ring slides against the teflon dielectric ring. (C) The rotating coaxial capacitors are shielded with copper housings and connected to the impedance matching network using coaxial structures. A pulley system controlled by a stepper motor is used to control the angle. The top pulley is shown attached to the right pivot. The right and left pillars are mechanical supports made of Delrin and used to anchor the structure to the probe body.

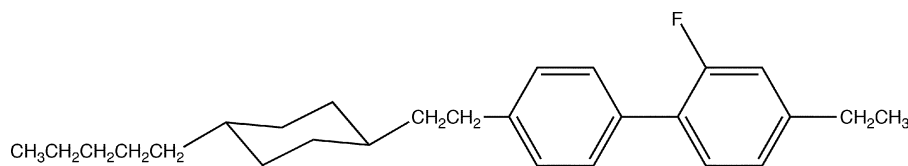
is a modified Alderman-Grant resonator, which combines several desirable features. This type of coil was originally designed to minimize the electric field in the sample region and reduce sample heating due to dielectric loss. This is an important consideration when working with biological samples with high salt concentration, as we will in future applications. Unlike a solenoid coil, this resonator produces a transverse field, such that the magnitude of  $B_1$  is independent of its orientation relative to  $B_0$ . In order for VAS experiments to be successful for a range of sample conditions, it is critical to be able to access the full range of angles. It is important to be able to apply strong decoupling fields when the sample is spinning close to parallel with the main magnetic field, where the dipolar couplings are at a maximum. Another advantage of this coil geometry is its high RF homogeneity over a region 4 mm in length.

In order to have a reasonable filling factor, the coil must fit closely around the sample and reorient with it during each experiment. This represents one of the major challenges in building VAS

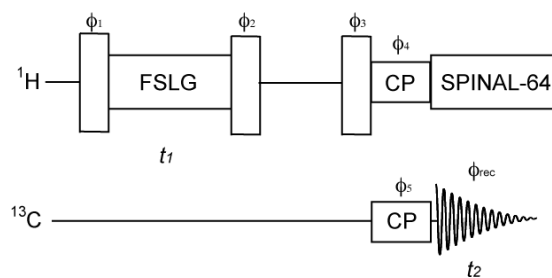
hardware; the moving coil must be connected with the static RF circuit in a manner that provides reproducible operation, does not wear out with repeated use, and does not generate noise or tuning instability due to stray reactances upon reorientation. Other designs have used sliding contacts<sup>[19]</sup> or flexible leads.<sup>[20]</sup> We address this problem using capacitive coupling. The sample coil is connected on each side to a copper ring, which is surrounded by a ring of teflon dielectric material, and then by another copper ring. Each of these coaxial capacitors makes a resonant circuit with the coil, such that the entire structure acts as a double-tuned probe circuit. Each channel is then impedance matched to 50  $\Omega$  using coaxial tuning tube structures. The frequencies used in this probe, which is used in our 11.7 T magnet, are 500 MHz (proton) and 125.7 MHz (carbon). When the coil reorients, the capacitance remains constant because of the cylindrical symmetry about the rotation axis. This eliminates changes in tuning during angle switching. Also, because there are no sliding contacts or flexible leads, the mechanical stress on the probe during long experiments with many hops is minimal.

The coaxial capacitors on each side are housed in a copper shield to minimize stray reactances and connected to the rest of the circuit by transmission line structures. The reorientation is performed using a pulley attached to one side of the circuit. This pulley is connected by Kevlar strings to another pulley at the bottom of the probe, and controlled by a stepper motor (General Electric/Fanuc PowerMax 11). The stator, which uses 4-mm rotors, bearings and drive assembly from Varian Inc, in Fort Collins, CO, is assembled around the coil.

As an initial demonstration, we performed HETCOR experiments on a model system using different spinning angles. We measured the  $^1\text{H}$ - $^{13}\text{C}$  dipolar coupling and anisotropic chemical shift at each angle and correlated these different experiments by linear projection. The sample is a mixture of ( $1$ - $^{13}\text{C}$ )  $\text{CH}_3\text{CN}$  and ( $2$ - $^{13}\text{C}$ )  $\text{CH}_3\text{CN}$  dissolved in the organic liquid crystal I52 (Fig. 3), which has a stable nematic phase at room temperature.<sup>[21]</sup> The concentration of each labeled species was 6.8% (v/v) in a total sample volume of 18  $\mu\text{l}$ . The magic angle is calibrated by observing the vanishing of proton homonuclear splitting in a standard sample ( $\text{CH}_3\text{CN}$  in this case). Other angles are measured relative to this using the calibration of the stepper motor, whose angular precision is within  $\pm 0.1^\circ$ . Figure 4 represents the HETCOR sequence without carbon decoupling. During the  $t_1$  period, the proton magnetization evolves in the presence of frequency shifted Lee Goldberg (FSLG) homonuclear decoupling.<sup>[22,23]</sup> No inversion pulse is used on the carbon channel so that the heteronuclear dipolar coupling is retained. During the optional delay after  $t_1$ , the magnetization is stored along the z-axis and proton spin diffusion allows equilibration of longitudinal magnetization. The magnetization is then transferred to carbon by cross polarization before signal detection begins. SPINAL-64 decoupling is applied during the detection period to remove heteronuclear dipolar coupling.<sup>[24]</sup>



**Figure 3.** Structure of the nematic liquid crystal solvent I52.



**Figure 4.** The HETCOR sequence with sample spinning at an arbitrary angle. The phase cycle is  $\phi_1 = (y, -y, -x, x)$ ,  $\phi_2 = (-y, y, x, -x)$ ,  $\phi_3 = (y, -y, -x, x)$ ,  $\phi_4 = (x, -x, y, -y)$ ,  $\phi_5 = (x, -x, y, -y)$ ,  $\phi_{\text{rec}} = (x, -x, y, -y)$ .

## Results and Discussion

The  $^{13}\text{C}$ - $^1\text{H}$  HETCOR in the case of the acetonitrile sample spinning at  $40.2^\circ$  is displayed in Fig. 5. Since we spin the sample off the magic angle during the entire experiment, the indirect dimension contains information about the anisotropic proton chemical shift and heteronuclear dipolar couplings, while the direct dimension represents the anisotropic carbon chemical shifts. The peak at 1.68 ppm corresponds to methyl carbon and that at 105.17 ppm corresponds to nitrile carbon. Both of these values are anisotropic chemical shifts. The observed splitting between the directly bonded carbon-proton pair is 548.0 Hz and the splitting between the carbon-proton pairs separated by two bonds is 137 Hz. After correction with the scaling factor, the actual values of heteronuclear couplings are 949 and 237 Hz, respectively. This type of experiment, demonstrated here on a set-up sample, provides the information necessary to get distance and orientational restraints for molecules in strongly oriented environments.

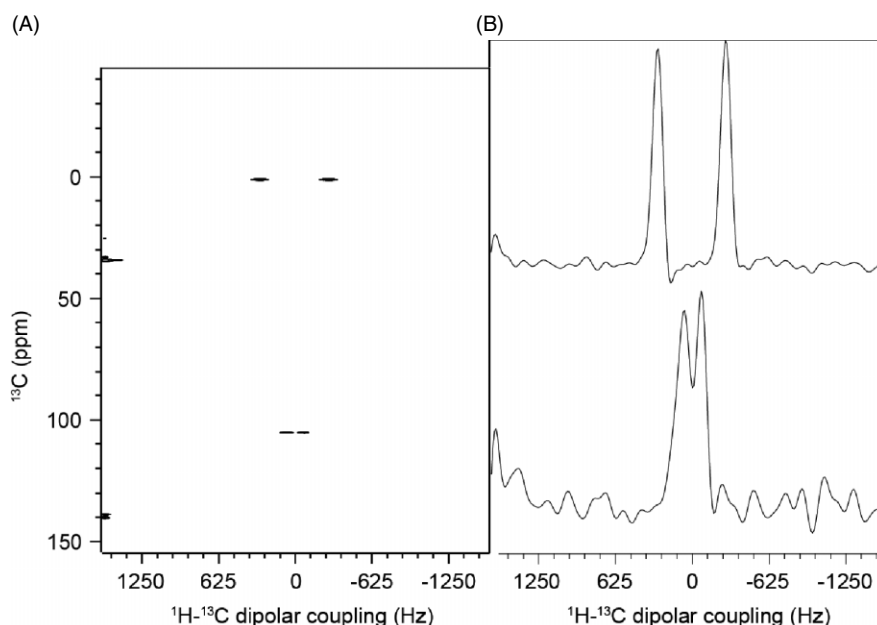
In the case of complex molecules, isotropic chemical shifts are also helpful for the assignment process. They can be obtained by acquiring spectra at several different angles and extrapolating from the angular dependence of chemical shift anisotropy, using the following relation:

$$\sigma(\theta) = \sigma_{\text{isotropic}} + \sigma_{\text{anisotropic}} \times P_2(\cos \theta) \quad (1)$$

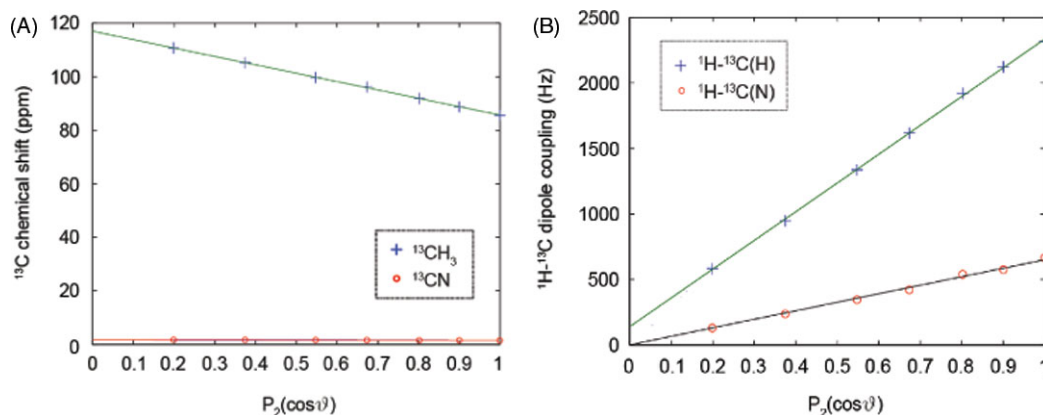
In this Eqn,  $P_2(\cos \theta) = (3 \cos^2 \theta - 1)/2$  with  $\theta$  representing the spinning angle, while  $\sigma_{\text{anisotropic}}$  and  $\sigma_{\text{isotropic}}$  correspond to the anisotropic and isotropic chemical shifts, respectively. As mentioned previously, VAS can also help to separate the contribution of dipolar coupling from J coupling, because the heteronuclear coupling has a similar dependence on the angular factor.

$$D(\theta) = J + D_{\text{dipole}} \times P_2(\cos \theta) \quad (2)$$

When the splitting value in the indirect dimension is plotted as a function of  $P_2(\cos \theta)$ , the slope of the curve gives the dipolar



**Figure 5.** The 2D spectrum (A) and 1D slices of coupled HETCOR (B) with 800 Hz sample spinning at 40.2. The sample is a mixture of (1-<sup>13</sup>C) CH<sub>3</sub>CN and (2-<sup>13</sup>C) CH<sub>3</sub>CN dissolved in organic liquid crystal I52, with the concentration of each labeled species at 6.8% (v/v). The total sample volume is 18 μl. For simplicity, the proton carrier frequency is set on resonance to the methyl chemical shift value. <sup>13</sup>C 90° time is 15.1 μs and <sup>1</sup>H 90° time is 11.8 μs. During *t*<sub>1</sub> period, a Lee Goldberg spin lock field of 50.81 kHz is applied to achieve homonuclear decoupling. The spectral width in the indirect dimension is 3.176 kHz, while the spectrum width in the direct dimension is 25 kHz. SPINAL-64 decoupling is applied at 21.2 kHz during signal detection. Other important experimental parameters are: *t*<sub>1 max</sub> = 15.10 ms, *t*<sub>2 max</sub> = 20.48 ms, the relaxation delay is 2 s, 16 scans are acquired for each FID, 64 FIDs are acquired for the whole spectrum. TPPI<sup>[25]</sup> is employed for frequency discrimination in the indirect dimension. Because of the application of FSLG homonuclear decoupling sequence, the apparent splitting in the indirect dimension is scaled by  $\sqrt{3}$  relative to the actual coupling value. Experiments are performed on 500 MHz Infinity CMX spectrometer.



**Figure 6.** (A) The linear dependence of chemical shift on the angular factor  $P_2(\cos \theta)$ . (B) The linear dependence of dipolar coupling on the angular factor  $P_2(\cos \theta)$ . The coupling values plotted here have been corrected with the scaling factor.

coupling (unscaled by sample spinning) while its intersection with *y* axis represents the *J*-coupling value. This is the principle of VACS<sub>Y</sub>.<sup>[26]</sup>

Figure 6 demonstrates the linear extrapolation of the isotropic chemical shift and heteronuclear couplings from spectra measured at different angles. In Fig. 6(A), the methyl carbon has almost constant chemical shift due to its small chemical shielding anisotropy. In addition, the chemical shift of the carbonyl carbon displays a linear dependence on the angular factor  $P_2(\cos \theta)$ , with the line intersecting with the *y* axis at 116.9 ppm, corresponding to the nitrile carbon isotropic chemical shift value. In Fig. 6(B), the curve for the adjacent coupling has a slope of 2193 Hz and an intersection of 140 Hz, while the curve for long-range coupling

has a slope of 666 Hz and an intersection at almost zero. This is because the two-bond *J* coupling between the carbon–proton pair is so small that it could not be resolved with our current spectral resolution. Note that all the coupling and shift values are measured with an error of  $\pm 5$  Hz arising from the error in the angle measurement.

## Conclusion

Variable angle spinning dipolar local field experiments are performed on our newly developed contactless resonator VAS probe with contactless resonator. For acetonitrile dissolved

in organic liquid crystals, heteronuclear dipolar coupling and isotropic chemical shift are extrapolated by correlating the spectra obtained at different angles. These proof-of-principle experiments establish the feasibility of using this hardware and methodology to obtain structural information in strongly oriented liquids. Future applications of VAS technology will include the measurement of long-range dipolar couplings in molecules strongly aligned in liquid crystals or membrane proteins embedded in lipid bilayers.

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