

# FastMAS and UltraFastMAS

Applications and experimental considerations

# **Application Note**

# Authors

Allen R. Palmer, David Rice, Siegfried Hafner, and Mircea Cormos Agilent Technologies, Inc. 5301 Stevens Creek Boulevard Santa Clara, CA 95051 USA Recent advances in spinning have made MAS experiments possible with rotation speeds between 40 and 65 kHz. For organic samples, the primary benefit from this technology is the linewidth reduction and suppression of spin diffusion brought about by averaging of the proton-proton dipolar interaction. In experiments such as direct proton observe or solid-state HETCOR, this averaging allows for spectra to be collected comparable to those which formerly required both spinning and experimentally difficult multiple-pulse homonuclear decoupling sequences. With MAS above 50 kHz, spin diffusion between protons is largely suppressed on a timescale of < 50 ms, allowing relaxation studies to be conveniently performed on individual protons in single or multiple dimensional experiments.

# Introduction

High resolution NMR in the solid state is complicated by a variety of anisotropic line broadening mechanisms. In some instances, these mechanisms may allow additional information to be obtained on a sample, but if the desired result is a simple liquids like spectrum then experimental and instrumental methods must be used to narrow the typically broad lines. In most cases, the method will achieve this line narrowing by averaging over time the molecular interaction that is causing the line broadening. To effectively average a molecular interaction, the timescale averaging must be fast as compared to the typical decay time caused by that interaction.



The largest interactions causing line broadening in solids fall into four basic categories. These are:

- 1. Chemical shift anisotropy
- 2. Heteronuclear dipolar coupling
- 3. Homonuclear dipolar coupling and
- 4. Quadrupolar interactions

These interactions have widely varying magnitudes, and over the years, different techniques have been developed to deal with each of them. Table 1 shows some of these interactions and where appropriate, the methods commonly used to average them.

Chemical shift anisotropy (CSA) for most part can be effectively managed using moderate speed magic angle spinning (MAS). This interaction does not vary with time (an inhomogeneous mechanism), so under MAS the positions of sidebands vary with speed, but the residual linewidth is a constant. Rotational resonances and the positions of sidebands may be a complicating factor, especially at high magnetic field, but a high quality spectrum may generally be obtained using conventional hardware.

In normal organic compounds, the source of line broadening with the largest magnitude is the dipole-dipole interaction. Heteronuclear dipolar interactions may be effectively and easily averaged using RF decoupling techniques, but using decoupling to average homonuclear dipolar interactions is far more problematic. Multiple pulse sequences (for example, CRAMPS) have been used for this purpose, but these sequences are instrumentally difficult to perform, and the intensities and residual line widths of resonances tend to reflect experimental imperfections rather than any underlying physics. Another method of eliminating the proton homonuclear dipole interaction is isotopic dilution, for example, deuterating a sample to a level at which protons become sufficiently weak. This method often provides the best proton linewidths but requires special sample preparation. Also, any integrations of spectra are only as accurate as the isotopic enrichment process, and correlation experiments are disrupted.

These difficulties have led researchers to increasingly faster MAS to average dipolar interactions in solids. Toward this end we have developed spinning systems designed for the routine collection of MAS and CP/MAS data at spinning speeds up to 65 kHz.

## **Experimental**

All data was taken on Agilent consoles at 600 MHz proton frequency using either an Agilent FastMAS or UltraFastMAS probe. All chemicals used were purchased from Aldrich and used without further preparation.

#### Physical appearance of the modules

The two spinning systems used for these experiments are similar to larger versions that are familiar to users of the Agilent Pencil modules (Figure 1). As in other Agilent spinning modules, the drive energy is delivered using a conical stator into which a similarly conical drive tip is seated. The action of the spinner creates a vacuum between the drive tip and the stator using the Bernoulli principle, eliminating the need for a cap over the upper end of the stator. Two ceramic air bearings, above and below the sample, provide stability for the rotor. Each air bearing has ventilation to the outside world both above and below. The rotor itself features non-protonated sample spacers near the sample for background reasons, and a drive cap and back end cap of a much harder material to withstand the spinning forces. Physical parameters of the two rotors are summarized in Table 2.

 Table 1.
 Summary of the Largest Interactions Causing Line Broadening in Solids. Of These Interactions, Only Chemical Shift Anisotropy is Proportional to the

 Applied Field so for all Other Mechanisms; Resolution is Improved with Higher Field Magnets

Interaction	Magnitude	∞ to Bo?	Typical solution
Chemical shift anisotropy	<sup>13</sup> C: 200 ppm <sup>1</sup> H: 20 ppm	Yes	Slow or moderate MAS
Heteronuclear dipole interaction	<sup>13</sup> C – <sup>1</sup> H: about 15 kHz	No	(Hetero-nuclear) decoupling
Homonuclear dipolar interaction	<sup>1</sup> H — <sup>1</sup> H: 50 — 100 kHz	No	CRAMPS Isotopic dilution (that is, partial deuteration) Very fast spinning
Quadrupole interaction	10's of Hz to many MHz	No	1 <sup>st</sup> order: (fast) MAS 2 <sup>nd</sup> order: DOR and ΜΩΜΑS



Figure 1. Cutaway of the spinning module. The two modules are similar in shape but vary slightly in dimensions. The 1.6 mm module is shown.
 Legend: A) Stator. B) Air Bearings. C) Temperature chamber.
 D) Temperature baffle. E) Sample Area.

#### Table 2. Rotor Descriptions for Two Spinning Modules

Module type	Rotor diameter	Maximum speed	Minimum speed	Sample volume
FastMAS	1.6 mm	45 kHz	5 kHz	8.1 µL
UltraFastMAS	1.2 mm	65 kHz	8 kHz	1.2 μL

#### **Thermal considerations**

With spinning speeds above 40 kHz, the heating from the applied RF field can generally be minimized by the use of very low power techniques for decoupling. Frictional heating from the action of the spinner is a far more difficult problem for which no perfect solution exists. In the absence of external air flow, the magnitude of this heating can exceed 50 °C for either the FastMAS or the UltraFastMAS when running near maximum speed. According to both theory and practice, the heating of a rotating cylinder is proportional to the square of the rotation rate, and this system (as well as other Agilent systems) follows this law closely.

Since this heating cannot be eliminated, steps have been taken to allow for accurate calibration of the difference between applied air and the actual sample temperature. If a calibration is to be reproducible, the sample temperature must be insensitive to environmental conditions such as the ambient laboratory temperature, and must also be insensitive to minor operational variations in the spinner performance. For example, the air bearing flow rate will vary slightly depending on the wear condition of the drive tip, sample mass, and sample imbalance within the rotor but it would be impractical to establish a separate temperature calibration curve for each possible condition. Lastly, the magnitude of the calibration should not vary widely with the set temperature for an experiment.

This isolation is accomplished by means of a temperature chamber separated from the air bearings by a pair of temperature baffles very tightly enclosing the temperature controlled area. The controlled temperature gas maintains a positive pressure within the chamber in order to prevent any mixing of the temperature controlled gas and the air bearing exhaust.

Figure 2 shows the typical calibration of the UltraFastMAS rotor as a function of temperature rise versus spinning speed. The data is nearly independent of actual sample temperature; rather, this is a value that should be added to the set temperature of the controller.





- Temperature rise with no VT airflow.
- Temperature rise with 60 L/min airflow.

# **High Resolution Proton Spectra**

#### Simple one dimensional spectra

A variety of techniques has been used to collect high resolution spectra of protons in the solid state (Table 3). Each of these has advantages and disadvantages, but each requires either special equipment or time-consuming preparation.

Table 3.	Common	Techniaues	for	Collectina	Hiah	Resolution	Proton .	Spectra	in	Solids

Technique	Advantage	Disadvantage				
CRAMPS	Better linewidths than fast MAS with low field instruments	Setup is tedious and imprecise				
		Quantitative results are difficult to impossible				
		Not practical for 2D homonuclear correlation spectra				
		Problematic at high field				
		Scaling of the chemical shift (which can be non-uniform over larger frequency offsets and depends on the experimental conditions)				
		Artifacts (failure of quadrature detection in standard multiple pulse techniques, center signal, offset dependent narrowing)				
Isotopic dilution	The best linewidths	Must deuterate the sample				
		Sample invasive technique				
		Using deuteration mostly prevents quantitative results				
		Not useful for most 2D applications				
Fast MAS alone	No special sample preparation	Quantitation is dependent on lineshape issues				
	Simple experimental setup					
	Better quantitation than other techniques	Very small sample volumes (can also be an advantage)				
	Higher sensitivity than CRAMPS					
	Artifact-free resolution					
	Suitable for high-field applications					

The analytical treatment of multiple-spin (homogeneously) broadened lines under fast MAS, as well as simulations by various researchers, predict that the proton line broadening from dipolar mechanisms (and thus its sensitivity) should be proportional to the spin rate. This prediction has been experimentally confirmed on various rigid solids spinning up to 70 kHz. At 70 kHz, the linewidths from typical organics are generally on the order of 300–500 Hz, that is, around 1 ppm on a 400 MHz instrument and 0.5 ppm at 800 MHz.

Figure 3 shows the proton spectrum of two simple compounds, alanine and monoethylfumaric acid (*trans*-CO<sub>2</sub>H-CH:CH-CO<sub>2</sub>-et), taken at spinning speeds between 10 and 70 kHz. As the line narrows with spinning speed, a corresponding increase in sensitivity equal to the line narrowing may be observed. Users should note that some of the increased sensitivity also comes from improvement in a broad region in the baseline near the resonances at lower spinning speeds. This broadening is not distributed evenly among the peaks, so accurate integration of proton lines is problematic with spinning speeds below approximately 50 kHz.





Tycho and Ishii have showed that this sensitivity advantage at high field and high speed is sufficient that indirect (that is <sup>1</sup>H) detection under fast MAS gives a significant advantage in <sup>15</sup>N sensitivity over a standard CP spectrum. The full 2D version of the indirect detection approach might thus replace direct HETCOR in many cases, similar to the HSQC experiment in liquids.

Due to the presence of multiple line broadening mechanisms, the extrapolation of the linewidth to infinite spinning speed does not always yield a highly narrowed line. The causes of this are inhomogeneous mechanisms such as imperfections in the crystals, natural isotropic chemical shift distributions, higher order quadrupolar couplings, and scalar couplings. Figure 4 shows the linewidths in spectrum 3(B) plotted as a function of the rotor period. Similar results have been observed by Samoson and by Gan.



Figure 4. Linewidths of the four resonances in ethylfumarate plotted against the rotor period. ◆ CH<sub>3</sub> group, ■ CH<sub>2</sub> group, ▲ CH group, ■ COOH group.

### Proton relaxation and spin diffusion in fast spinning solids

Solid-state NMR spectroscopists generally make the assumption that due to spin diffusion the <sup>1</sup>H spins can be approximated as a single homogeneous spin temperature reservoir. With spinning above 40 kHz this is not valid, as the spin diffusion rate is approaching the relaxation rate in many samples. Therefore, any experiment to measure either rate constant must consider each proton species separately, and must also take both processes into account. Spin diffusion is not eliminated with 70 kHz MAS, but between chemically different, but nearby, protons (for example,  $CH_2 \Leftrightarrow CH_3$  in an isolated ethyl group) it is suppressed to rate constants on the order of 200–800 ms. In many cases, this is still faster than T<sub>1</sub> processes, so spin diffusion heavily affects relaxation curves.

Figure 5 shows  $T_1$  relaxation at 62 kHz (inversion recovery data) for ethylfumarate, demonstrating the relative independence of the proton relaxation of the various chemically different protons under these conditions. Note particularly that the slowly relaxing COOH proton shows little response until magnetization has had time to diffuse from the hydrophobic end of the molecule.



Figure 5. Inversion-recovery  $T_1$  data for the four resolvable peaks in ethylfumarate at 20 °C and spinning at 65 kHz.  $\diamond$  CH<sub>3</sub> group,  $\blacksquare$  CH<sub>2</sub> group,  $\blacktriangle$  CH group,  $\blacksquare$  COOH group.

In principle, these data are sufficient to calculate every  $T_1$  and every diffusion coefficient in the molecule if one assumes that longer range diffusion constants are negligible. In practice, however, the slower relaxing groups are sufficiently dominated by spin diffusion that the error bar in the  $T_1$  measurement would be too large for the measurement to be useful. The diffusion coefficient can be listed with a reasonable precision. Figure 6 shows the measured diffusion constants in ethylfumarate spinning at 65 kHz.



Figure 6. T<sub>1</sub> relaxation and spin diffusion constants, in seconds, which may be calculated from T<sub>1</sub> data in Figure 5. Allowing for longer-range diffusion constants leads to redundant solutions unless other measurements are used.

Spin diffusion may also be measured directly through exchange spectroscopy. With this sequence, (similar to classical NOESY), cross peaks are formed by either chemical exchange or spin exchange. Figure 7 shows the exchange spectrum of ethylfumarate at two different spinning speeds with identical 200 ms mixing times.



 Fig. 7. Exchange spectra of Ethylfumarate. A) Spinning speed is 40 kHz.
 B) Spinning speed is 65 kHz. In both cases, mixing time is 500 ms. In addition to the improved resolution at high speed, spin exchange is noticeable suppressed, especially between non-adjacent resonances. A spectrum taken with 40 kHz spinning and a 100 ms mixing time produces cross peak intensities nearly identical to B, suggesting that the long range cross peaks in A are due to serial spin migration rather than direct exchange.

# **Carbon Spectra With High Spinning Speed**

### Sensitivity issues

As spinners and sample volumes become smaller, sensitivity becomes a greater issue. At high field this is somewhat compensated by improved coil geometry. For a solenoidal sample coil, the total length of the coil wire, and thus the number of turns on that coil, is limited by the quarter-wavelength of the highest frequency the coil must support. Thus, as the diameter of the coil drops, the number of turns on the coil and the coil's efficiency as an antenna is improving. In the case where a limited amount of sample is available, this improved coil geometry gives rise to a significant increase in sensitivity. Figure 8 shows the absolute sensitivity for several Agilent solids probes, as well as the sensitivity versus sample volume for the same probes. It can be seen from this figure that the 1.6 mm probe has nearly the sensitivity of a standard 3.2 mm probe, and that the 1.2 mm probe is approximately a third of that value.



Figure 8. Comparison of the <sup>13</sup>C sensitivity coresponding to different rotor systems as the rotor diameter increases.
Shows the increase in sensitivity as rotor diameter(and thus volume) increases.
▲ Shows the increase in sensitivity/mg sample as the rotor

size decreases. Experimental conditions: Sample is HMB, 4 scans, CP with contact time power ramp, 5 kHz spinning, apodization matched to natural linewidth.

## **Decoupling under fast spinning MAS**

Traditional high power CW decoupling performs poorly in high speed spinning systems due to interference between the decoupling nutation frequency and the spinning frequency. Due to the small size of the rotor, the probe can deliver 300 kHz of decoupling power to the sample, but even 300 kHz decoupling is insufficient for reasonable linewidths in simple 1D spectra. Techniques such as TPPM and XiX are effective at 300 kHz, but this power density is unacceptable for many samples. Alternative approaches to this brute force high power decoupling techniques are thus needed.

With fast spinning, the dipolar coupling between protons and carbon has been significantly reduced (Figure 9). As a decoupling technique, however, the standalone MAS result is poor as compared to the result achievable using 65 kHz CW RF decoupling in a slowly spinning sample. In addition to the residual dipole coupling, the carbon linewidth also includes scalar interactions such as J-coupling and crossterms

between chemical shifts. These require RF irradiation in some suitable form, but decoupling techniques may now be designed to focus only on the much smaller couplings that remain under fast spinning conditions



Figure 9. Comparison of 60 kHz CPMAS spectra of alanine acquired with different decoupling techniques.

The simplest such technique uses conventional CW decoupling, but with the rf amplitude low as compared to the spinning speed. Spinning with 65 kHz, this low rf amplitude is as effective as 300 kHz high power decoupling, but is still inferior to moderate decoupling with low spinning speed. The XiX scheme improves the performance of this low power cw approach by applying a 180 degrees phase modulation, where the modulation frequency is optimized with respect to the rotor period. Although this is an improvement, it requires setup and is not as effective as either high power XiX or TPPM.

As an alternative to these continuous rf irradiation decoupling techniques, we have found that efficient decoupling is possible even for rigid solids using a train of 180 degrees <sup>1</sup>H pulses synchronized to the rotor speed (Figure 10). The phase shift between pulses should be around 90 degrees, but is not a critical parameter as long as it is neither 0 degrees nor 180 degrees. We refer to this as <u>PI Pulses Synchronized</u> decoupling (PIPS). In addition to lowering the average power, this cycle is simple to set up, requiring only knowledge of the approximate 180 degrees pulse width and the spinning speed. Figure 9 shows spectra of alanine using several decoupling sequences for comparison.





As the spinning speed increases, it becomes unnecessary to apply the 180 degrees pulse each rotor cycle, and effective decoupling is achieved with a single pulse every two to four cycles. In addition to lowering the average power, this increases the bandwidth of effective decoupling and decreases the sensitivity to the pulse width. This improves the spectrum to a level achieved by windowless decoupling sequences such as TPPM.

#### **Cross polarization under fast spinning**

Cross polarization in solids acts through the dipolar couplings, so as these couplings are modulated with fast spinning efficient cross polarization becomes difficult. Additionally, as in decoupling, the high power required for traditional methods is damaging to many samples. Fortunately, however, techniques exist which use the rotor speed to minimize these issues.

It is well established that mechanical rotation affects the CP process and a spinning term needs to be included to the static match condition,  $\omega_{1H} = \omega_{1C} \pm n \omega_R$ . Thus, as the spinning speed increases, the difference between the power levels also increases. Spinning very high speeds can be used to drive down the proton power to levels as low as 4 kHz while maintaining a sufficient spinlock (Figure 11 A).

We have found that greater efficiency and more general magnetization transfer can be achieved by using a series of discrete pulses on protons during the spin locking sequence (Figure11 B). The pulses are synchronized to the rotor period. Spectra using these sequences are shown in Figure 12.



Figure 11. Low power CP sequences for high speed spinning.
A) Classical CP, but with proton power reduced to low values using the spinning frequency term.
B) Pulsed Magnetization transfer.
The spinlock pulse of conventional CP is replaced by a series of

rotorsynchronized pulses. The match is adjusted between the rotor term  $\omega_R$  and the carbon rf amplitude  $\omega_{1\chi}$ .



Figure 12. Cross polarization methods for High Speed Spinning.
A) Conventional CP at 280/220 kHz.
B) Low power CP using fields of 60 / 4 kHz for carbon and protons, respectively.
C) Pulsed CP. The pulses are synchronized with the rotor period, each pulse is 350 ns with a power level of approximately 300 kHz.

#### The HETCOR experiment

HETCOR in solids is a 2D experiment in which the <sup>1</sup>H dimension is encoded during the evolution period after which the magnetization is transferred to the detection nucleus by cross-polarization. The resulting signal is detected under heteronuclear decoupling conditions (Figure 13). As a result, a two dimensional correlation spectrum between <sup>1</sup>H and <sup>13</sup>C is obtained. With moderate speed MAS, the proton resolution is generally insufficient to yield meaningful data, therefore multiple pulse homodecoupling must be applied during the evolution period and to both channels during the crosspolarization period. This experimental complication has prevented solidstate HETCOR from gaining general acceptance outside of advanced solid-state NMR laboratories.





With FastMAS, the dipolar averaging from spinning makes unnecessary any RF driven homodecoupling, and the experiment now attains the simplicity of liquids heteronuclear correlation experiments. Setting up such an experiment accurately requires no more effort than to set up the corresponding CPMAS experiment.

Figure 13 shows a low power version of the HETCOR sequence suitable for fast spinning rotors. This is a combination of low power CP and PIPS decoupling, both of which have been previously discussed. In this case, we have used a CW version of the cross-polarization as the pulsed version of CP gives rise to slightly longer-range couplings. Figure 14 shows the HETCOR spectrum of ethylfumarate taken using the CP sequence illustrated in Figure 13.



Figure 14. HETCOR spectrum of Ethylfumarate. Note that there is a 0.1 ppm difference between the chemical shifts of the two CH groups in the proton dimension, which is not resolvable in solids using one-dimensional spectra.

Tycho and Ishii showed in 2000 that indirect (for example, <sup>1</sup>H) detection under fast MAS gives a significant advantage in <sup>15</sup>N sensitivity over a standard CP spectrum. The full 2D version of the indirect detection approach might thus replace direct HETCOR in many cases, similar to the HSQC experiment in liquids.

## Conclusions

Direct observation of proton experiments are practical with fast MAS. Linewidths are approximately proportional to the reciprocal of the spinning rate, and for normal organics fall in the range of 300–500 Hz when spinning at 60 kHz. This is approaching the linewidth of a typical CRAMPS experiment, but lacks the experimental artifacts common to that technique. This freedom from artifacts simplifies many two dimensional homonuclear experiments that were previously impractical under multiple pulse conditions.

At 60 kHz MAS, spin diffusion between two nearby but chemically different protons has been suppressed to a time constant which generally falls between 100 and 800 ms. This is on the order of many proton  $T_1$  rates, so in simple relaxation experiments, these processes typically compete. For simple experiments on heteronuclei, the improved coil geometry in small coils allows fast spinning probes to offer sensitivity comparable to that in conventional probes using rotors as large as 3 mm, but with only a fraction of the sample volume. For simple carbon spectra, FastMAS is usually unnecessary, as moderate spinning and decoupling provide adequate line narrowing. HETCOR experiments, are greatly simplified with faster spinning. In these types of experiments, multiple pulse homodecoupling can be eliminated, and the experiment becomes simple enough to be routine. Experiments similar to the familiar liquids HSQC experiment are also practical.

# Appendix A

### Techniques for handling very small samples

Agilent has developed a set of sample handling tools to assist in the handling of tiny rotors and small amounts of sample. The purpose of these tools is that once a rotor has been inserted into the tool, each part of the rotor is held in the tool and need not be directly touched by the operator. Shown in Figure 16 are several views of this tool.

Figures 15 A and B show the rotor in a configuration for sample packing. The rotor itself is held in a collet, and a funnel part covers the end of the rotor. The funnel includes an internal step such that the hole through the funnel matches the inside diameter of the rotor.

Figure 15 C shows the tool for removing the end cap. The rotor is placed in the same collet as in Fig. 15 A and a clamp is applied to the rotor cap. A jam nut can then be slowly unscrewed to apply even pressure on the cap forcing it to pull straight from the rotor sleeve. A similar tool (not pictured) is used to press the end cap in place once the sample has been loaded.



Figure 15. Rotor packing tools for small rotors. A and B show views of the rotor as it is held for sample packing, while C shows the mechanism for removing the endcap. a, Collet. b, Sample funnel. c, End cap clamp. d, Jam nut.

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