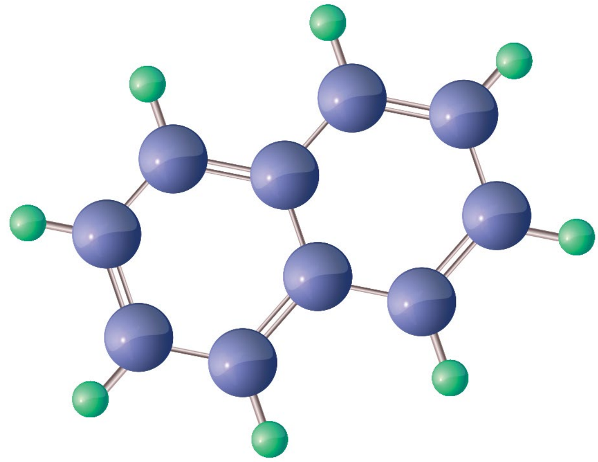

Cross Polarization up to 111 kHz MAS & More

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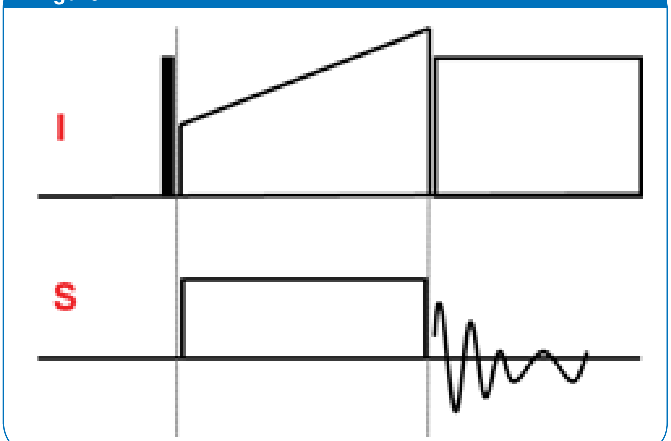
Cross Polarization (CP) is the primary tool in solid state NMR, including magic angle spinning (MAS) NMR, to transfer magnetization between pools of nuclear spins. The nuclei can be like or unlike nuclei. If transferring magnetization from abundant nuclei, like ^1H spins to rare nuclei, CP helps to enhance the magnetization of the rare nuclei, for example ^{13}C, in two ways. First, and most familiarly, the enhancement is proportional to the ratio γ^{1H}/γ^{13C}, of the gyromagnetic ratios γ^{1H} and γ^{13C}. Secondly, it benefits from the typically much faster longitudinal spin relaxation T1 of the ^1H spins (1), which allows a shorter recycle delay when signal averaging or collecting multi-dimensional data. In the other case, CP between rare nuclei, CP is essential in order to establish heteronuclear correlations in multi-dimensional experiments.

CP consists of two 'contact' pulses (figure 1) simultaneously applied to the two pools of spins I and S. The one that carries the polarization to the other nuclei is called the spinlock pulse and requires transverse magnetization be available at the same phase as the pulse. The other contact pulse develops magnetization if its amplitude is correct and fulfills the Hartman Hahn condition (1,2). In order to create an initial condition of transverse magnetization in the I-spin pool, a 90° pulse is applied before the I-spin contact at 90° out of phase compared to the spinlock pulse. Any of the contact pulses can have an amplitude modulation (see right).

Figure 1



Displays the basic cross polarization experiment. Our experiment used the amplitude ramp on the I spin and always from low to high amplitude, as shown, although equivalent performance may be obtained by instead inverting its slope and/or applying to the S spin with constant amplitude on I. The open rectangle following the ramped pulse on channel I represents any decoupling pulse sequence, while the filled rectangular pulse is a 90° excitation pulse for I spins, which are ^1H spins in our case.

Although CP has long been in common use for MAS NMR, the more recent pushes to very high rotation rates (above 40 kHz), or for cross polarization between rare spin-½ nuclei at lower rotation rates, requires more attention to experimental details like amplitude modulation, spin nutation frequencies on the nuclei pools and their relation to the mechanical sample rotation. In this note, we present experimental procedures for a quick and successful optimization of polarization transfer using CP for various experimental goals by qualitatively aligning our experimental findings with theoretical predictions.

Cross polarization (1) was extensively discussed for various regimes of rotation rates, for example by B. Meier (2), by M Baldus et al. (3) for weak dipolar couplings, which exist between rare spin1/2 nuclei like ^{13}C and ^{15}N, the so called specific CP, to the ultra-fast regime of 65 kHz and higher (4) and for second order CP, by A. Lange et al (5). As our focus is on the experimental rather than the theoretical aspects, the above mentioned references can be used as starting point for those who are interested, studying the theoretical background. While discussing CP results based on such theoretical expectations, we still need to consider the influence of the rf coils in our probes on the CP result. Multiply tuned coils always have an inhomogeneous rf field over the length of the rf coil and different rf profiles for each frequency channel. One can easily imagine that rf balance as well as rf inhomogeneity affect the CP experiment and thus will influence the specific choice of experimental elements in order to achieve optimum polarization transfer (6).

Under MAS, we know three major CP conditions. These are the zero quantum CP (ZQCP) (2, 4), double quantum CP (DQCP) (2, 3, 4), and second-order CP (SOCP) (2, 5). The Hartmann Hahn condition for ZQCP is

$\omega_I = n\omega_{MAS} + \omega_s$, [1]

with $\omega_I = 2\pi f_I$ and $\omega_S = 2\pi f_S$ being the angular spin-nutation frequencies of spins I and S. The mechanical angular frequency of rotation about the magic angle is $\omega_{MAS} = 2\pi f_{MAS}$ with f_{MAS} being the MAS rotation rate The parameter n is a non-zero integer, ±1, ±2. In contrast, the condition applying for DQCP experiment is

$\omega_I + \omega_S = \omega_{MAS} * n$, [2]

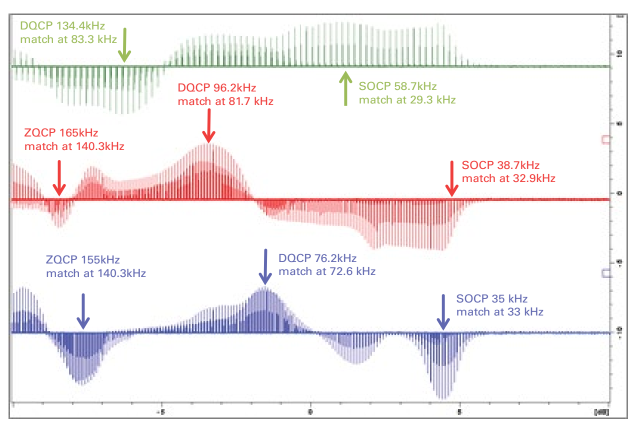
and for SOCP it is

$\omega_I = \omega_S$.   [3]

Typically, we need $\omega_I < \omega_{MAS}$ for effective SOCP. A further requirement for any CP is that the magnitude of the rf pulses (i.e., the matched nutation frequencies f_I and f_S) may not be an integer multiple m of the MAS rate f_{MAS} nor a low-order fraction of the MAS rate $f_{rf} = \frac{f_{MAS}}{m}$ with $m = 1,2$ and 3 [See Refs. (2, 4, 5)]. This requirement is necessary to avoid rotary resonance, a homonuclear recoupling condition (10) that leads to magnetization losses for the spin-locked magnetization (5). The spin lock field should be either at or above $f_{S,I} \geq 2.5 * f_{MAS}$. If, at very high MAS rates, the above required f_{rf}-fields cannot be reached without damaging the hardware, we must use lower rf-fields. In such a case, rf fields at $f_{S,I} < f_{MAS}$ or $f_{I,S} > f_{MAS}$ $f_{S,I} = a * f_{MAS}$ with $a = \frac{2}{5}, \frac{3}{5}, \frac{3}{4} < \frac{1}{3}$ $or\ \frac{2}{3}$ using ZQCP, DQCP or SOCP work well [See also figure 3 in Ref. (5).]. Such qualitative considerations are similarly important in the case of double cross polarization (DCP), where a minimum f_{1H} for ^1H-decoupling field of $f_H \geq 2.5 * f_{MAS}$, is required during CP between ^{13}C and ^{15}N to avoid interference between ^1H decoupling field and the matched ^{13}C or ^{15}N rf fields for regular MAS rates up to 60 kHz. At MAS rates at or above 100 kHz, proton decoupling during C-N CP step is typically avoided.

The magnitude of the magnetization transfer and thus the final signal intensity depends on the magnetic rf field distribution in the NMR coil (6, 7). In order to deal with inhomogeneous B1-fields experimentally, we use amplitude modulations for one of the contact pulses (8, 9). We generally have to assume that both, adiabatic CP conditions (9) and B1-homogeneity contribute to the signal intensity during a CP step (6, 7). For our purpose, the highest signal amplitude is experimentally relevant. Therefore, we content ourselves being qualitatively aware that amplitude ramped pulses effectively increase the experimentally accessible sample volume under the rf coil (6) while they also provide adiabicity for magnetization transfer (9) and thus influence the magnitude of the magnetization transfer of CP. We base our choice of a ramp on empirical evidence namely a wide range optimization of the ^1H contact pulse amplitude for a variety of amplitude modulated rf pulses for a defined constant amplitude rf pulse on the other nucleus. These empirical results are, of course only strictly valid for the probe used, for the measured ratio of MAS rate to rf field in a narrow range of MAS rates.

Figure 2



Optimization trails of glycine spectra for various ramps: 50% (green), 30% (red) and 10% (blue) with the following CP condition at 111 kHz: ¼ $= \frac{\omega_{MAS}}{2\pi}$ for ^{13}C constant amplitude contact pulse. The ^1H spinlock pulse amplitude is varied from -10 dB to 10dB. Contact pulse length is 2 ms. The green, blue and purple arrows indicate ZQCP, DQCP and SOPC, respectively.

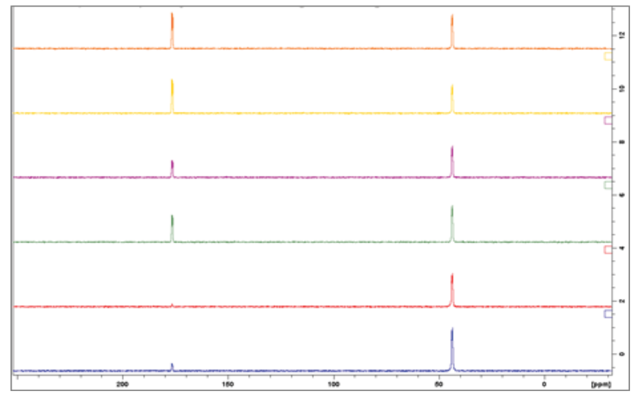
We studied ^{1}H contact pulses with 50%, 30% and 10% linear amplitude ramps. A 50% amplitude ramp is achieved by a linear change of the pulses rf amplitude from 50% to 100% for a given power level. Likewise, 30% or 10% ramp implies a linear amplitude change from 70% to 100% or from 90% to 100% rf amplitude, respectively, for a given power level. It is instructive to relate the f_{rf} amplitude variation to rf power changes and we find that the 50% ramped pulse represents a 6dB rf power change; the 30% pulse a 3.09dB change and the 10% ramp a 0.91dB rf power change. Of course the ramp can be inverted and applied for 100% to 50 % and likewise for the other amplitude ramps we discussed. It is still debated if the time orientation, which is the ramp up or ramp down, is experimentally significant. Pulse programs permit to alternatively employ the amplitude modulated pulse on the X channel instead on the ^{1}H channel. We did not test that option in the current study.

In the following we report on the ^{13}C signal intensity of the two ^{13}C resonances of uniformly ^{13}C and ^{15}N enriched glycine for a series of CP matching conditions by stepwise decreasing the ^{1}H f_{rf} power of amplitude ramped contact pulses. We incremented the attenuation of these rf pulses in steps of 0.1 dB for the 30% and 10% ramps from -10 dB to +10 dB (20.33 kHz) for a fixed ^{13}C field of f_{rf} = 27.75 kHz at a rotation rate of f_{MAS} = 111 kHz on our 0.7 mm CPMAS probe. For the 50% amplitude ramped pulse, we find a negative maximum signal intensity for both carbon resonances at -6.4dB (Figure 2 green trace). This is equivalent to 134.3 kHz at 100% amplitude of the ramp's contact pulse. We calculate for this maximum that the Hartman Hahn match would be for a DQCP condition at 83.25 kHz, which is well below the midpoint of the amplitude ramp at 62% of the calculated rf field amplitude. The positive intensity maximum of the green trace in Figure 1 is located at 0.8dB and identifies a SOCP process, which leads to a selective enhancement of the C$^{\alpha}$-resonance.

For the 30% amplitude ramped pulse, we find one maximum at -3.5dB. That maximum represents a 96.17 kHz ^{1}H rf field and thus turns out to be a DQCP condition with effective 81.7kHz on ^{1}H and 29.2 and 28.6 kHz on C^{O} and C^{a}, respectively, if we consider effective fields for the 9 kHz and 7.1 kHz offsets of these resonances from ^{13}C carrier. We find a negative maximum at 4.4 dB with a ^{1}H field of 38.73 kHz which is almost equal to the effective fields for the 2 carbon resonances of 28.6 kHz (C$^{\alpha}$) and 29.2 kHz (C^{O}). Interestingly, we only find the C$^{\alpha}$ resonance enhanced in this SOCP condition (red spectrum in figure 3).

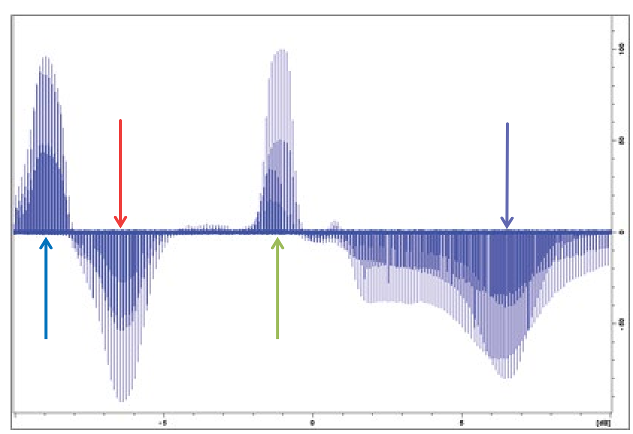
For the 10% ramp we observe the most signal amplitude variations and find the first significant negative maximum at 155.97 kHz, identifying as a ZQCP event. The next positive maximum is at 76.39 kHz, which is a DQCP. The selective SOCP gives the negative maximum at 38.73 kHz. The SOCP provides the largest C$^{\alpha}$-resonance intensity not only for the 10% ramp, but also one compared to all other amplitude ramped CP experiments we studied.

Figure 3



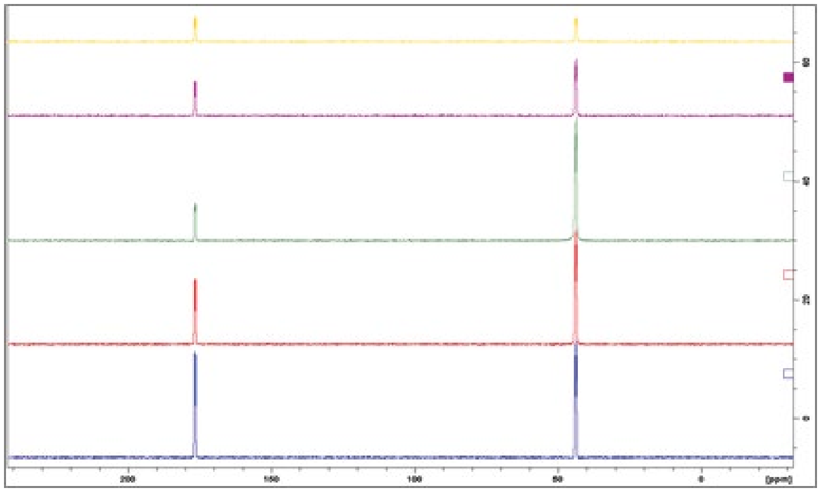
Spectra of the discussed extrema for 10% ramp, (blue SOCP, yellow DQCP and orange ZQCP) for 30% ramp (red SOCP, green DQCP) and 50% ramp (purple DQCP). The blue spectrum has the highest Ca resonance of all experiments presented while the orange ZQCP spectrum presents the highest overall integral intensity of both resonances. The green spectrum has the second highest absolute intensity for Ca while the CO resonance is below the intensities for CO on the yellow and orange spectrum.

Figure 4



The optimization trail of the glycine spectrum from -10 dB to +10 dB rf-power on a 10% linear amplitude ramp ^{1}H contact pulse of 2 ms duration, while the spinlock pulse amplitude on ^{13}C was 2/3rd of 100 kHz MAS rate, at 66.67 kHz. We find the ZQCP at 173.85 kHz (blue arrow) mid value for 10% ramp, the DQCP n=2 condition at 129 kHz (red arrow), the SOCP condition at 69.3 kHz (green arrow) and the DQCP, n=1, condition at 20.92 kHz (purple arrow).

Figure 5

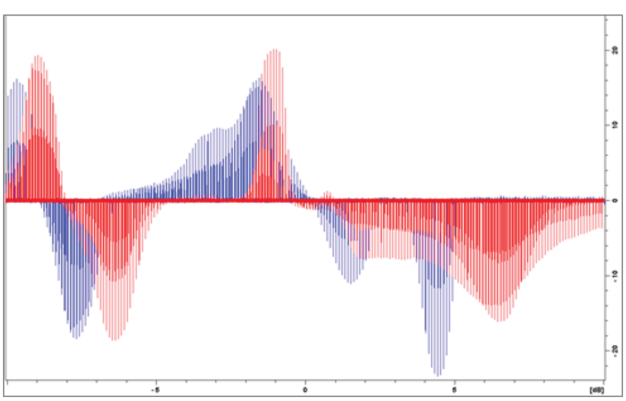


The glycine spectrum at the optima pointed out in figure 4 with the spinlock pulse on ^{13}C at 2/3rd of 100 kHz MAS rate, which is a 66.67 kHz rf-amplitude. The ZQCP lies at 173.85 kHz (blue spectrum), the mid value for 10% ramp, the DQCP n=2 condition at 129 kHz (red spectrum), the SOCP condition at 69.3 kHz (green spectrum) and the DQCP, n=1, condition at 20.92 kHz (purple spectrum). We find the overall highest intensity is obtained for the ZQCP condition, while the most selective CP step with the overall highest C$^{\alpha}$ amplitude is obtained for the SOCP condition.

Next, we change the ^{13}C rf-field and set f_{rf} = 66.67 kHz, which is 2/3rd of the MAS rotation rate of 100 kHz where we expect a DQCP condition for ^{1}H at 33.33 kHz, a SOCP condition at 66.67 kHz and a ZQCP condition at 166.67 kHz. With a 10% ramp we find that ZQCP delivers the overall best CP intensities for both resonances together. The SOCP condition provides the strongest overall C$^{\alpha}$ signal.

Comparing the two sets of experiments with 10% amplitude ramps but with the $f_{13C}=\frac{1}{4}f_{MAS}$ at 111 kHz MAS and the one with $f_{13C}=\frac{2}{3}f_{MAS}$ at 100 kHz MAS, indicates that the higher rf-field gives a better polarization transfer (Figures 4 and 6). These observations are in qualitative agreement with spinlock measurements by A Lange et al (5). We found on our 700 MHz spectrometer that $^{13}C\{^{1}H\}$ CP at 111 kHz worked best with $f_{13C}=\frac{3}{4}f_{MAS}$ and the n=1 ZQCP condition at 194 kHz on ^{1}H pulse (data not shown).

Figure 5



Comparison of the optimization traces between -10 dB to +10 dB rf-power for a 10% linear amplitude ramp ^{1}H contact pulse of 2 ms, while the spinlock pulse amplitude on ^{13}C was at 66.67 kHz rf amplitude, which is 2/3rd of 100 kHz MAS rate(red trace) and at 1/4th of 111 kHz MAS rate, which is 27.75 kHz (blue trace). We clearly see the improved signal intensity for the higher ^{13}C spinlock condition, while the SOCP provides a quite similar CP yield for the C$^{\alpha}$-resonance.

To summarize our observations, in the case of CP at high rotation rates of 42 kHz and upward, SOCP, ZQCP and DQCP can be employed in order to achieve efficient magnetization transfer, see table 1. For selective excitation, SOCP appears to be the method of choice with rf fields $\frac{1}{4}f_{MAS}<f_{rf}<\frac{1}{3}f_{MAS}$. ZQCP provides the highest possible signal, if the rare nucleus contact pulse is between $\frac{2}{3}f_{MAS}\leq f_{rf}\leq\frac{3}{4}f_{MAS}$ and $f_{^{1}H}=\omega_{MAS}+\omega_{^{13}C}$. Polarization transfer between rare nuclei follows these observations in general as well but requires some additional attention and will be dealt with in an upcoming note.

Table 1

ZQCP						
MAS Rate	**a**	**x Field**	**^{1}H Field**	**^{1}H Field 10% ramp**	**^{1}H Field 30% ramp**	**^{1}H Field 50% ramp**
kHz		**kHz**	**kHz**	**kHz**	**kHz**	**kHz**
42	1.75	73.5	115.5	121.6	135.9	154.0
62.5	0.75	46.9	109.4	115.1	128.7	145.8
100	0.75	75.0	175.0	184.2	205.9	233.3
111	0.75	83.3	194.3	204.5	228.5	259.0
120	0.75	90.0	210.0	221.1	247.1	280.0
140	0.75	105.0	245.0	257.9	288.2	326.7
DQCP n=1						
MAS Rate	**a**	**x Field**	**^{1}H Field**	**^{1}H Field 10% ramp**	**^{1}H Field 30% ramp**	**^{1}H Field 50% ramp**
kHz		**kHz**	**kHz**	**kHz**	**kHz**	**kHz**
42	0.60	25.2	16.8	17.7	19.8	22.4
62.5	0.60	37.5	25	26.3	29.4	33.3
100	0.60	60	40	42.1	47.1	53.3
111	0.60	66.6	44.4	46.7	52.2	59.2
120	0.60	72	48	50.5	56.5	64.0
140	0.60	84	56	58.9	65.9	74.7
DQCP n=2						
MAS Rate	**a**	**x Field**	**^{1}H Field**	**^{1}H Field 10% ramp**	**^{1}H Field 30% ramp**	**^{1}H Field 50% ramp**
kHz		**kHz**	**kHz**	**kHz**	**kHz**	**kHz**
42	1.75	73.5	10.5	11.1	12.4	14.0
62.5	0.75	46.9	78.1	82.2	91.9	104.2
100	0.75	75.0	125.0	131.6	147.1	166.7
111	0.75	83.3	138.8	146.1	163.2	185.0
120	0.75	90.0	150.0	157.9	176.5	200.0
140	0.75	105.0	175.0	184.2	205.9	233.3
SOCP						
MAS Rate	**a**	**x Field**	**^{1}H Field**	**^{1}H Field 10% ramp**	**^{1}H Field 30% ramp**	**^{1}H Field 50% ramp**
kHz		**kHz**	**kHz**	**kHz**	**kHz**	**kHz**
42	0.75	31.5	31.5	33.2	37.1	42.0
62.5	0.6	37.5	37.5	39.5	44.1	50.0
100	0.4	40	40	42.1	47.1	53.3
111	0.4	44.4	44.4	46.7	52.2	59.2
120	0.4	48	48	50.5	56.5	64.0
140	0.4	56	56	58.9	65.9	74.7

Rf-field conditions at various MAS rates between 42 and 140 kHz or the ZQCP, DQCP and SOPC and the three amplitude ramps, 10% 30% and 50% that were studied. The ^{1}H Field column shows the calculated rf field while the following fields show the required rf field value for the 100% rf field value required for the ramp employed, 10%, 30% or 50% assuming that the optimum match is ad the mid position of the ramp, at 95%, 85% and 75%, respectively.

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