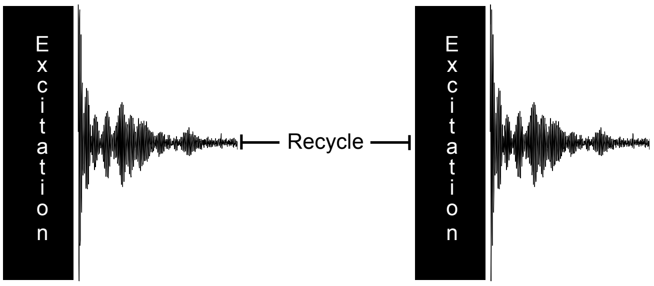
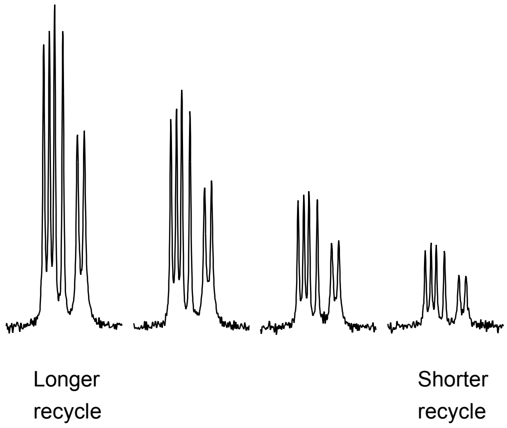
3.6 Choosing the Right Recycle Delay

Choosing the right recycle delay is the first step in any NMR experiment. Here, the recycle delay is defined as the time between the end of the data acquisition from one FID and the start of the next period of radio-frequency excitation (whatever that may be). It is during this delay that the excited sample returns to its equilibrium state and the optimum delay is sample dependent. The delay is called many things, but commonly, relaxation delay, recycle delay (or simply recycle) or pulse delay. Recycle delay (or recycle) is the recommended term.



The choice of recycle determines (among other things) the amount of signal detected. Too long a delay will result in wasted spectrometer time. Too short a delay can result in the loss of signal through saturation[1]. There is no limit to the longest delay that can be used (other than the spectroscopist's patience) but caution must be used with very short delays. In general, to protect the probe and sample, a safe policy is not to exceed a 20% duty cycle[2].



After the first excitation the sample returns to its equilibrium state at a rate $1/T_1$ where T_1 is the spin-lattice relaxation time-constant. Consideration of the equations that describe this process shows that to obtain the full signal after the second excitation, a recycle (t) of approximately $5\times T_1$ is required. A delay of this duration is necessary for any quantitative experiment[3]. For a heterogeneous sample, with multiple-component T_1 behaviour, a recycle of five times the longest T_1 should be used if accurate relative intensities are to be obtained.

At time $t=0$ $\langle M_z \rangle = M_0$ and $\langle M_x \rangle = 0$

After a 90° pulse $\langle M_z \rangle = 0$ and $\langle M_x \rangle = M_0$

At time t later $\langle M_z \rangle = M_0\left(1 - e^{-t/T_1}\right)$

The magnetisation still in the xy plane is $M_0 e^{-t/T_1}$

A pulse applied at time t gives $\langle M_x \rangle = M_0\left(1 - e^{-t/T_1}\right)$ ...(A)

t	$5\times T_1$	$4\times T_1$	$3\times T_1$	$2\times T_1$	$1\times T_1$
$\langle M_x \rangle$	$0.993\times M_0$	$0.982\times M_0$	$0.950\times M_0$	$0.865\times M_0$	$0.632\times M_0$

---

[1] In a saturated system there is no population difference between excited and ground states so no signal can be measured.

[2] Duty cycle: $\frac{\text{r.f. on-time}}{\text{r.f. on-time + r.f. off-time}} < 0.2$

[3] Assuming that the recycle is much longer than the acquisition time so that the latter can be ignored - almost always the case for solids.

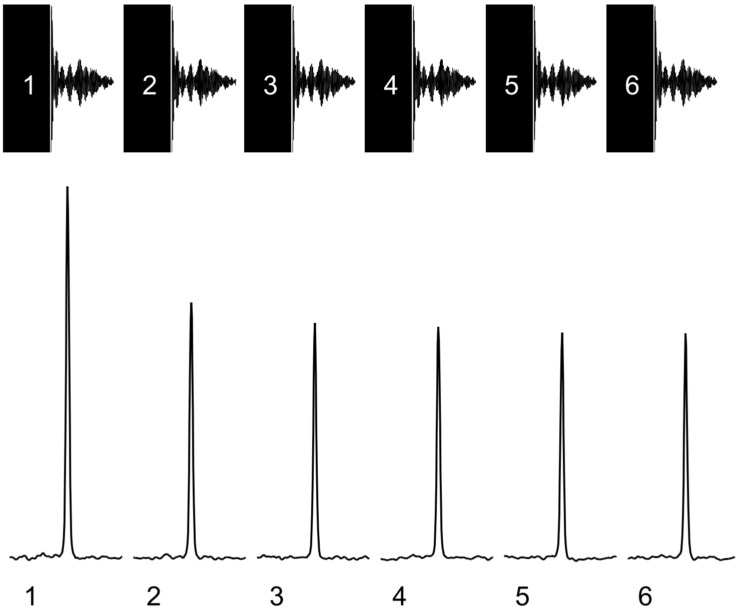
If we consider the situation a further time t after the second excitation we find that the magnetisation along the z-axis is the same as it was after the first period t. This situation must also arise after all subsequent t periods.

At time t after the second excitation

$$\langle M_z\rangle = M_0\left(1-e^{-t/T_1}\right)\left(1-e^{-t/T_1}\right)+M_0 e^{-t/T_1}\left(1-e^{-t/T_1}\right)$$

or $$\langle M_z\rangle = M_0\left(1-e^{-t/T_1}\right)$$

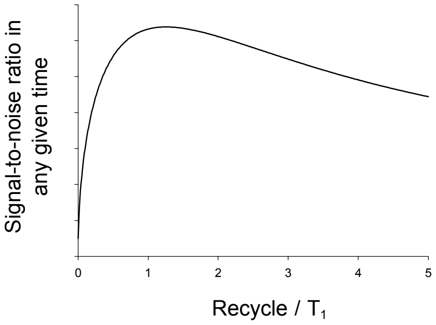
This can be demonstrated experimentally. Spectrum 1 (opposite) resulted from one excitation with the sample originally at equilibrium. This is the maximum amount of signal that can be obtained from one excitation (of the type used here). Spectrum 2 comes from the next FID alone. The recycle delay here is 0.8×T_1. Spectrum 3 comes from the next FID and so on. As predicted, the signal intensity generated by each excitation, after the first, is (approximately) the same.



For any given time the final S/N is determined by the balance between the recycle delay (t) and the number of repetitions (n). The best S/N is not obtained by waiting 5×T_1 for a fully relaxed spectrum. Instead, the optimum recycle delay is ~1.2×T_1. This is the delay to use for any experiment where quantitative signal intensities are not important; for example, any cross-polarisation experiment where other factors render the intensities non-quantitative anyway[4].

The table gives the results of five real experiments, of the same duration (40 s), but with different recycle delay/number of repetition combinations. It suggests our simple theoretical treatment is certainly good enough for choosing a recycle delay.

$$\frac{signal}{noise} \propto \frac{n}{\sqrt{n}} M_0\left(1-e^{-t/T_1}\right)$$



Repetitions	Recycle / T_1	S/N
8	5	114
12	3.33	127
32	1.25	154
80	0.5	157
392	0.1	88

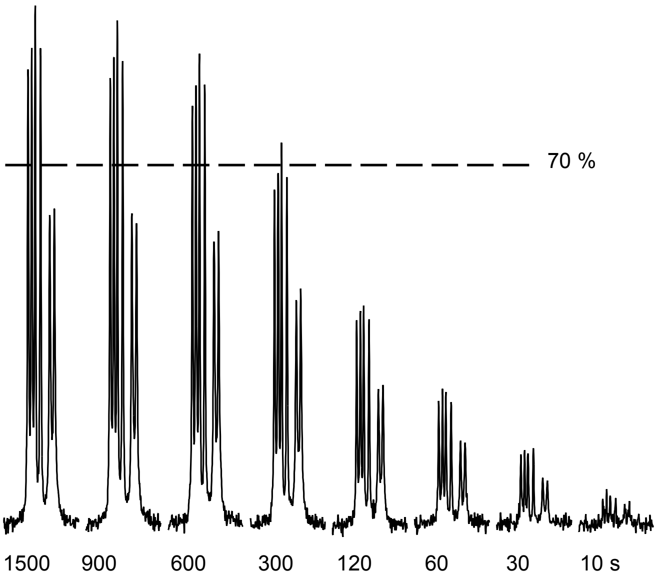
---

[4] Remember that is for a 90° pulse. For a DP experiment an alternative is to use a small ("Ernst") angle pulse according to the recipe:

$$pw = \cos^{-1}\left(\exp\left(\frac{-t}{T_1}\right)\right)\times\frac{pw90}{90}$$

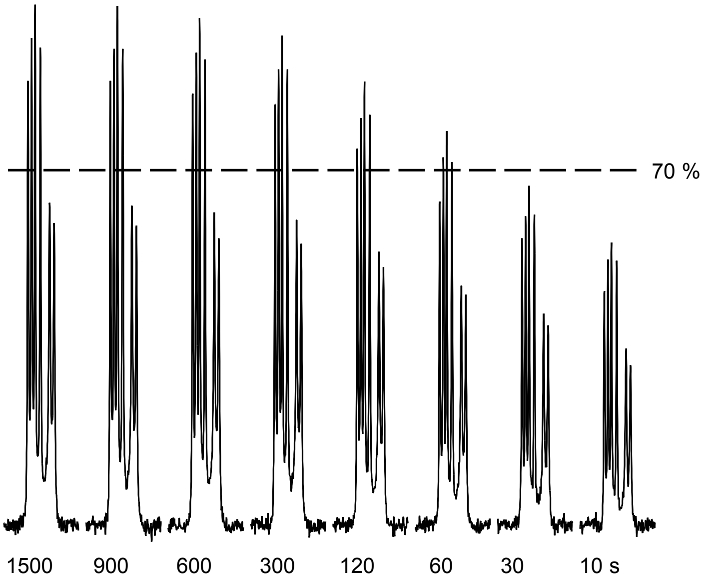
where, t is the chosen recycle and pw90 is the 90° pulse duration.

So, to choose the recycle delay we need T_1. That is, for a CP experiment we need $T_1^{(H)}$ or for a DP experiment $T_1^{(X)}$. If we do not want to measure T_1 properly we can get a good estimate of the value to use by "arraying" the recycle delay. The exact result is spectrometer dependent so see the footnote at the end of the section. Nevertheless, if the first two elements of the array give the same intensity then it is safe to say that both of the spectra are fully relaxed. If we want quantitative spectra and we are doing a DP experiment then we have the appropriate delay. If we want the best S/N in any given time then the optimum recycle is the delay that gives ~70% of this full intensity[5]. In this example $T_1^{(H)}$ is 226 s so we are faced with a recycle delay of ~280 s for a CP experiment.



For CP experiments with ^{1}H decoupling through the acquisition (so most of them) we can improve things. Adding a "flip-back" pulse on the ^{1}H channel allows us to "drive" the experiment faster. This extra pulse is a 90° pulse (with a duration appropriate to the r.f. field strength) of opposite phase to the first ^{1}H 90° pulse. The flip-back pulse returns to the z-direction any magnetisation that remains spin-locked at the end of the acquisition. This short-cut to equilibrium can circumvent the need for a long recycle.

The reduction in the optimum recycle delay can be dramatic. These spectra were obtained with the same recycle delays as the previous ones but with a flip-back pulse added to the pulse-sequence. With flip-back we could use a recycle delay of ~40 s so reducing the experiment time by a factor of seven.



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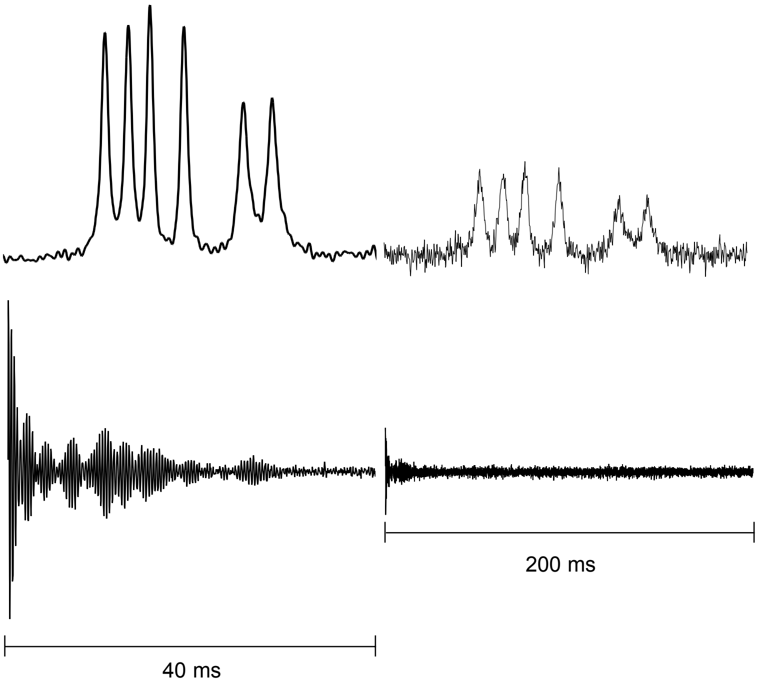
[5] This follows from $t = 1.2 \times T_1$ in equation (A)



Flip-back is effective when $T_{1\rho}^{(H)}$ > contact time + acquisition time. Only then will sufficient magnetisation still be spin-locked at the end of the acquisition. The longer $T_{1\rho}$ the better. [In this particular example $T_{1\rho}$ > 160 ms]. It is therefore important to choose the acquisition time with care. Here, the two spectra (plotted on the same scale) were obtained under conditions that differed only in the acquisition time. With 40 ms there is (a) no truncation of the FID and (b) little accumulation of noise after the signal has decayed. This is when flip-back is potentially at its most effective. If too long an acquisition time is used additional noise acquired in the FID results in extra noise in the spectrum. There is less signal because flip-back is less effective (less magnetisation is spin-locked at the end of the acquisition) and, with the recycle used here, signal is lost through saturation.

For flip-back to work at all the ^{1}H spin-lock must be uninterrupted. Any experiment in which the decoupling is turned off will make flip-back ineffective. This can explain lower than expected intensities in dipolar-dephasing experiments, for example. Other experiments where caution must be used in selecting a recycle delay (because they can involve an interruption to the decoupling) include the measurement of $T_1^{(X)}$, by the Torchia method, and $T_{1\rho}^{(X)}$. Flip-back also seems to be ineffective when two-pulse, phase modulated (tppm) decoupling is used.

Footnote

The exact appearance of an array of different recycle times depends on how the array is implemented on the spectrometer. The figures here all come from a VNMR pulse sequence on a Varian Unity Inova spectrometer where the recycle delay is executed first and the elements of the array simply run-on one after the other. We always run the longest delay first. For example, if we want to look at recycles of 10, 5, 2 and 1 s with four repetitions of each, the complete experiment looks like (ignoring any overhead or preacquisition delays):

(wait 10 s - acquire)$_4$ - (wait 5 s - acquire)$_4$ - (wait 2 s - acquire)$_4$ - (wait 1 s - acquire)$_4$

If we do the same thing under Spinsight on an Infinity Plus spectrometer we get:

(acquire - wait 10 s)$_4$ - (acquire - wait 5 s)$_4$ - wait 10 s - (acquire - wait 2 s)$_4$ - wait 10 s - (acquire - wait 1 s)$_4$.

This will produce a subtly different (perhaps more rigorous) result but the general principles in choosing a recycle still apply. The additional delay means, especially if you are testing very long values, doing the array the other way round (1, 2, 5 then 10 s) is more time-efficient although, again, the result may be slightly different to either of the other experiments.

3.7.1 Basic Cross-polarisation (Matching)

By basic cross-polarisation (CP) we mean cross-polarisation using "square" pulses. We will deal with different versions of the CP experiment in subsequent sections. Here we will assume that the CP is ^{1}H to ^{n}X. It could equally well be F⇨X or X⇨Y, the same principles apply.

It is worth a quick word on the advantages (and disadvantage) of CP (for H to X). The main advantage is that in a CP experiment, the recycle depends on T_1(H) and not T_1(X) and the former is usually much shorter than the latter so we can accumulate many more repetitions in any given time. There is also a signal gain associated with CP. The theoretical maximum gain is γ^H/γ^X so for low-γ nuclei this enhancement can equate to a substantial improvement in signal-to-noise ratios. Probe background signals also tend to be less important for CP (the plastics used in spinner housings can be perfluorinated so there can be no H-C CP signal, for example). The main disadvantage with CP is that relative signal intensities usually cease to be quantitative. However, that is a price worth paying for the majority of low-abundance nuclei.

In some cases, for abundant nuclei (^{19}F or ^{31}P, for example), the advantages associated with CP are not so important and here a CP experiment may be desirable for its ability to edit a spectrum in a particular way (more of this is later sections) or to establish relationships between nuclei (this would be the primary reason for doing X to Y CP).

Nucleus	$\gamma^H / \lvert\gamma^X\rvert$
^{19}F	1.06
^{31}P	2.47
^{119}Sn	2.67
^{13}C	3.98
^{29}Si	5.03
^{17}O	7.37
^{15}N	9.86
^{109}Ag	21.4
^{103}Rh	31.6

A short note on phase cycling: the pulse on the X-channel can generate a signal, just like any other pulse, indepenent of that arising from the cross-polarisation. We can arrange the phase cycling so that this signal does not contribute to the final spectrum:

90 (H)	x	x	-x	-x	
Spin-lock (H)	y	y	y	y	
Contact (X)	x	y	x	y	
Receiver	x	y	-x	-y	
Magnetisation arising from CP appears along	x	y	-x	-y	accumulated
Magnetisation arising from DP appears along	-y	x	-y	x	summed to zero

Reversing the phase of the proton 90° pulse is what we do when we talk about spin-temperature inversion in a CP context. When carrying out CP experiments it is, therefore, important to acquire multiples of the full phase-cycle, particularly so for high-abundance nuclei (such as ^{19}F and ^{31}P).

The standard CP pulse sequence is shown in figure 3.5.1. There are four "pulse" elements to the sequence, each, potentially, with its own B_1 field.

First, we apply a ^{1}H 90° pulse (with field $B_1^H(90)$). This rotates magnetisation from z to -y (say). Once it is along this axis we apply a pulse on y to keep it there. This is what we call the "spin-lock" (with field B_1^H(spin-lock)). With the spin-lock in place we apply a pulse on the X-channel (with field B_1^X(contact)). The time for which these two pulses are on together is the contact time. After the contact time we extend (possibly with an amplitude change) the H-irradiation to decouple H (with field B_1^H(decouple)) while we observe X on the other channel.

Cross-polarisation occurs efficiently when the H and X r.f. fields are "matched". Then $\gamma^H B_1^H = \gamma^X B_1^X$. The first step in setting up a CP experiment is to set the r.f. fields so that this condition is met. We will assume that this will be done using a standard sample so that we know the correct recycle and contact time to use and that we have good estimate for the duration of the ^{1}H 90° pulse. To set the match we can either vary B_1^X or B_1^H.

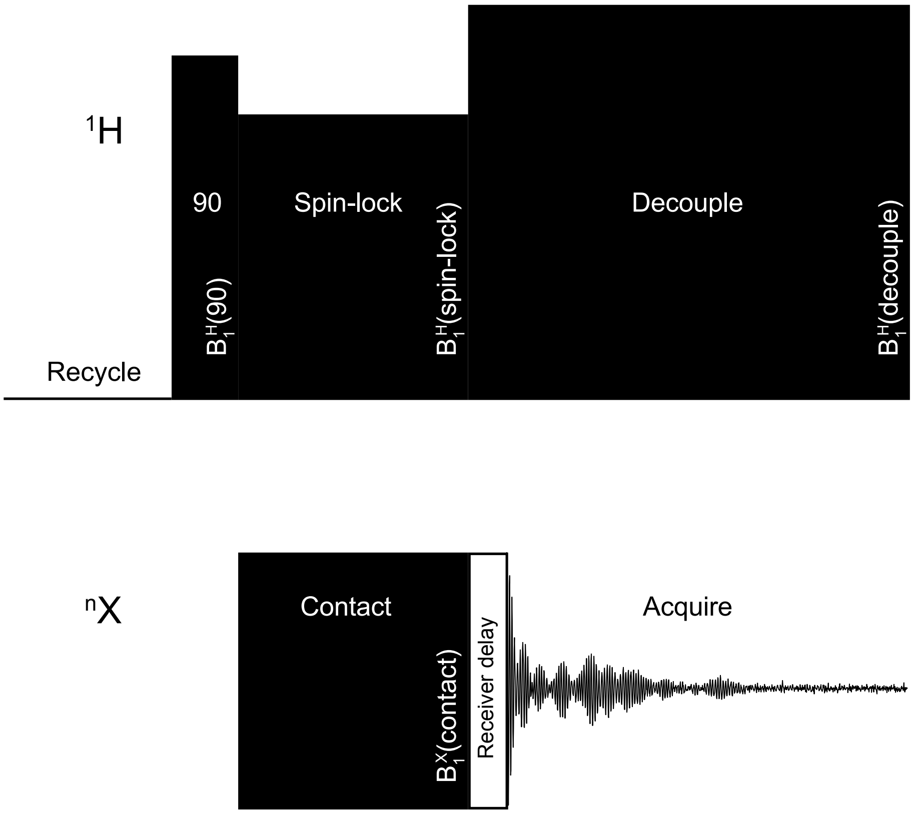


Figure 3.5.1 Cross-polarisation (^{1}H to ^{n}X) pulse-sequence.

B_1^H(spin-lock) is the safest to vary because probes have to cope with much less power on the H-channel (we know, for example, that γ^C is approximately four times γ^H, so for a match, B_1^C must be four times B_1^H). It is very important to know what the limits the probe you are using has with respect to r.f. power. Make sure you know these before you change any power settings.

If we want to vary B_1^H(spin-lock) we, ideally, need to have independent control over the amplitude of the preceding 90° pulse ($B_1^H(90)$) - otherwise we are changing two things at once. The alternative is to leave B_1^H(spin-lock) alone and vary B_1^X(contact). This must be done with more care and is not the recommended method. Whichever field is adjusted, it is a matter of changing the field strength until maximum signal is obtained - see figure 3.5.2.

Once you have found the match it is a good idea (whichever channel you adjusted) to check that the ^{1}H 90° pulse is set properly (vary the pulse duration until maximum signal is obtained - as in section 3.4). If you set $B_1^H(90)$ to equal B_1^H(decouple) then the 90° pulse duration gives you the field strength for the decoupling, similarly if $B_1^H(90)$ is set to B_1^H(spin-lock) you have the field for the CP step. In a matched experiment $P_{90}(H)$ (the ^{1}H 90° pulse) should equal $P_{90}(X)$. If you want to check $P_{90}(X)$ the easiest way is to add a pulse to the X-channel, immediately after the contact, as shown in figure 3.5.3. Recording the signal as a function of this pulse duration gives you $P_{90}(X)$ when you null the signal. Precisely locating a null is usually easier than finding an exact maximum so this method gives you an accurate method for determining the X-channel 90° pulse if you need it. It retains all the advantages of the CP experiment and is particularly useful if you are going to be switching between CP and DP experiments. It is best to be on- or near-resonance when you do this though.

The upper limit to the B_1 field strength depends on the probe (NEVER exceed it!). The lowest practical

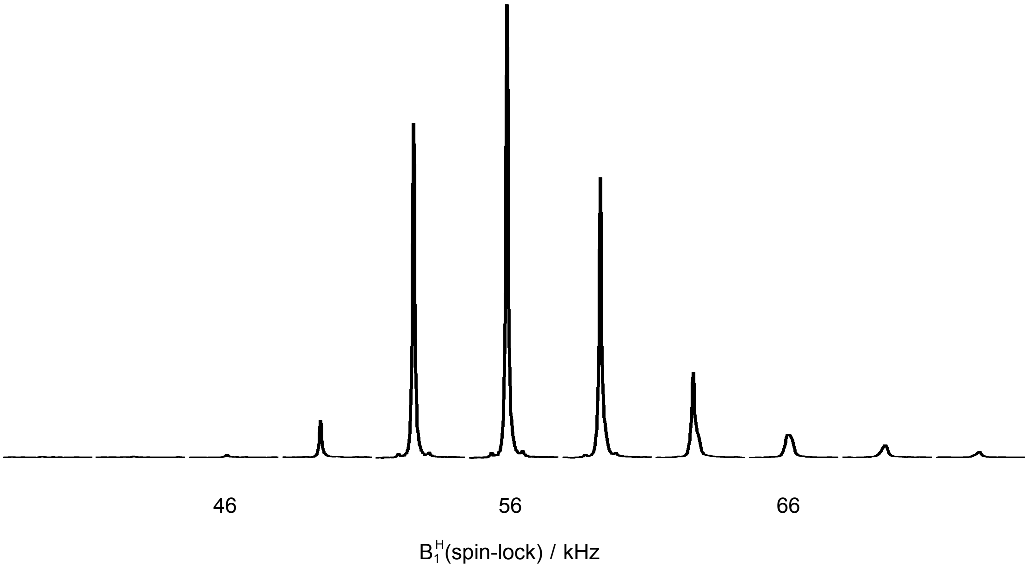


Figure 3.5.2. The high-frequency line from adamantane as a function of B_1^H(spin-lock). Note the poor lineshape for the “mis-matched” spectra.

field depends on the sample. For CP to be effective the magnetisation must remain spin-locked during the contact-time (neglecting, for the moment, the effects of $T_{1\rho}$) . At very low r.f. fields this may not happen. For effective spin-lock, B_1^H (in kHz) must be greater than the half-height linewidth from the static ^{1}H line. Few static linewidths exceed 40 kHz so any field greater than this is going to be adequate for most of the time.

One other factor that needs to be considered when matching is the spin-rate. Complications can arise at high spin-rates (see section 3.9) so we usually use relatively low spin-rates when we match with our standard samples. For example, for adamantane (^{13}C) and tetratrimethylsilylmethane (^{29}Si) we spin at around 1 kHz, with brushite ($CaHPO_4{\cdot}2H_2O$, ^{31}P), tetracyclohexyltin (^{119}Sn), and most other CP standards, we spin at 3 to 5 kHz.

Once established on a reference sample the match tends to be relatively insensitive to the sample (so a degree of mismatching can usually be tolerated). There are two exceptions: samples containing a lot of water can interfere with the r.f. enough to necessitate rematching on the sample itself. As mentioned in the last

Some basic relationships between field strength, voltage and power.

$$B_1 \propto \sqrt{\frac{P}{\nu}}$$

where P is the power in watts and ν is the frequency.

$$P = \frac{V^2}{400}$$

where V is the peak-to-peak voltage (at an impedance of 50 Ω).

$$V = 20\sqrt{P}$$

so 100 W = 200 V and 1 kW = 632 V. You have been warned!

$$B_1 = \frac{1}{4 \times P_{90}}$$

$$B_1 = \frac{2\pi}{\gamma}\,\frac{1}{4 \times P_{90}}$$

The r.f. field strength is often quoted as the “equivalent” to a frequency in kHz. P_{90} is the 90° pulse duration in μs. In “proper” units the magnetogyric ratio is needed. In most experiments B_1 is between 0 and 6 mT.

paragraph the use of high spin-rates can cause problems (and the definition of “high” is sample dependent) but we will come on to those in section 3.9. There are other factors that influence the efficiency of a CP experiment but we will discuss those in a later section as well.

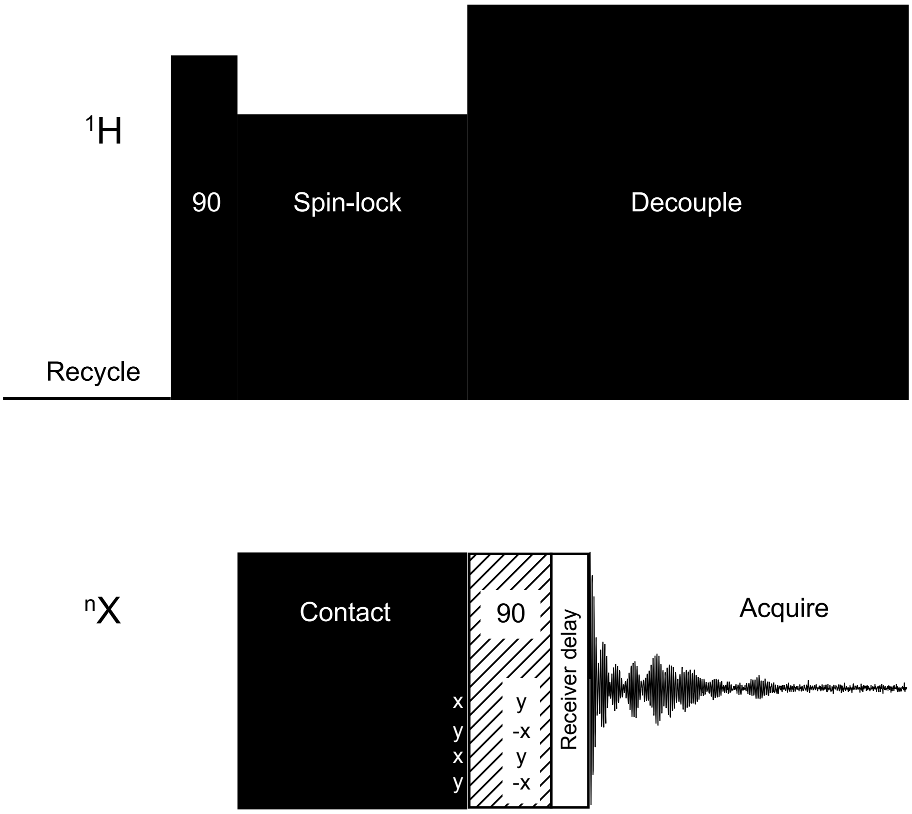
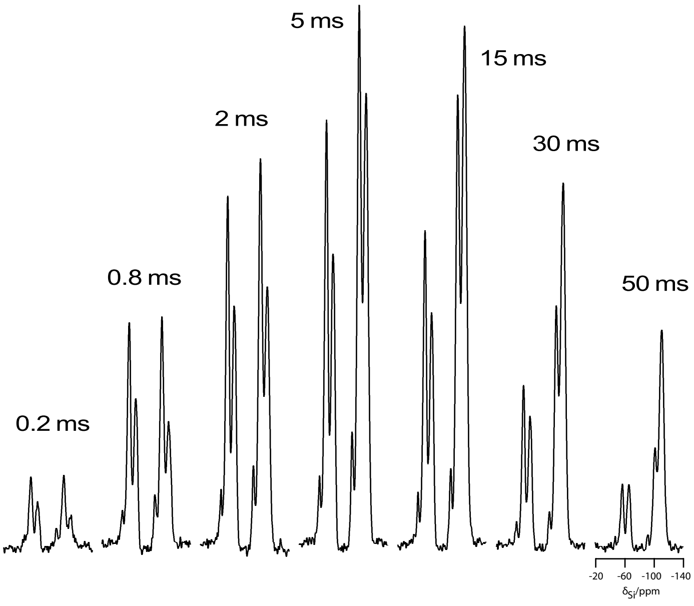


Figure 3.5.3 Cross-polarisation pulse-sequence with an extra pulse for calibrating $P_{90}(X)$.

3.7.2. Choosing the Right Contact Time

The contact time (or just contact for short) is the time during which the r.f. fields are applied simultaneously in a CP experiment - see figure 3.5.1. Choosing a contact time is best done after finding the right recycle delay, so that the effect of saturation does not obscure the effect of changing the contact time. If we are simply interested in generating the spectrum with the highest signal-to-noise ratio, choosing the contact is easy. It is just a matter of recording test spectra as a function of the contact time and choosing the one that gives most signal. It is easy if the spectrum contains just one signal or if all the signals behave in the same way. If they do not then you either have to record a series of spectra or use a compromise value that shows best the features you are interested in. This example, from an H to Si CP experiment, comes from an organically modified silicate. While the signals do behave differently the choice of contact, if we just want a spectrum that shows us what species we have present, is relatively easy to make: 5 ms here. It also beautifully illustrates the folly of reading too much into the relative intensities from a single CP spectrum!



Remember that you are putting the probe under more electrical strain if you use a long contact so you might have to reduce the field strengths if you do want to use a long one (greater than 10 ms, say, although it is relatively rare to need - or be able to use - a contact greater than this). In terms of obtaining a spectrum this is all there is to learn about the contact time. However, it is useful to know how and why the contact time varies, which is what the rest of this section deals with. See also the hint at the end of the section.

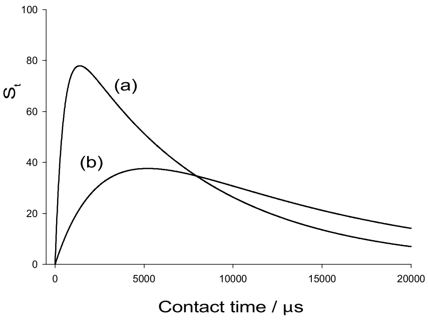
For a low-abundance nucleus (^{13}C, ^{15}N or ^{29}Si for example) or an effectively dilute spin (such as ^{31}P in many cases) the contact time dependence of the signal can be described by the following equation.

$$S_t = S_0\lambda^{-1}\ 1-\exp\ {}^{-\lambda t}\!/_{T_{XH}}\quad \exp\ {}^{-t}\!/_{T_{1\rho}^{H}} \qquad \text{where} \qquad \lambda = 1+\frac{T_{XH}}{T_{1\rho}^{X'}}-\frac{T_{XH}}{T_{1\rho}^{H}}$$

$1/T_{XH}$ is the cross-polarisation rate, $T_{1\rho}^{X'}$ is the spin-lattice relaxation time of the X nuclei in the rotating frame (under ^{1}H decoupling) and $T_{1\rho}^{H}$ is the proton spin-lattice relaxation time in the rotating frame.

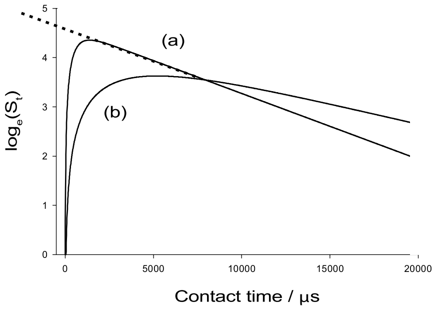
It is usual to make some simplifying assumptions so that λ ~ 1, then.

$$S_t = S_0\ 1-\exp\ {}^{-t}\!/_{T_{XH}}\quad \exp\ {}^{-t}\!/_{T_{1\rho}^{H}} \qquad ....B$$



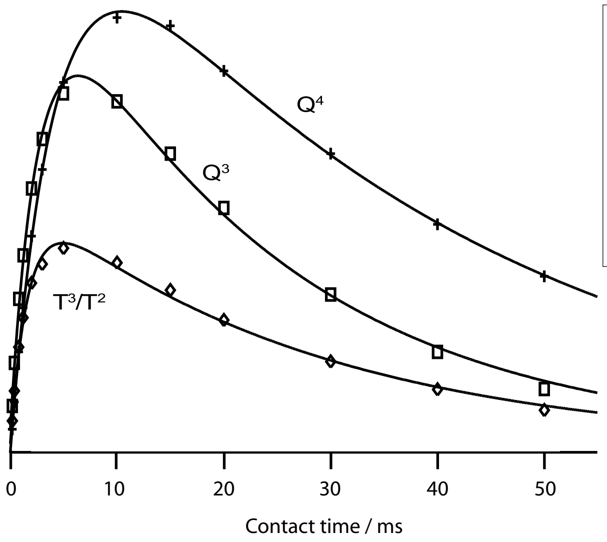
We have assumed here that the CP is from ^{1}H. The signal dependence on contact time can then often look like this. Here, T_{XH} is 0.5 and 3.5 ms and $T_{1\rho}^{H}$ is 7.5 and 12.0 ms for (a) and (b) respectively. Note, again, the deceptive intensities, these curves are for a 4:3 (a:b) population ratio.

Each curve has a rise time determined by T_{XH}. This rise is particularly dependent on the strength of the coupling between X and H so anything that lessens this (distance or motion for example) will reduce the CP rate. There is a real example of this later in the section. At the same time as the signal buildup, the ^{1}H magnetisation available for CP is reduced through $T_{1\rho}^{H}$ - this causes the eventual decay in the CP signal.

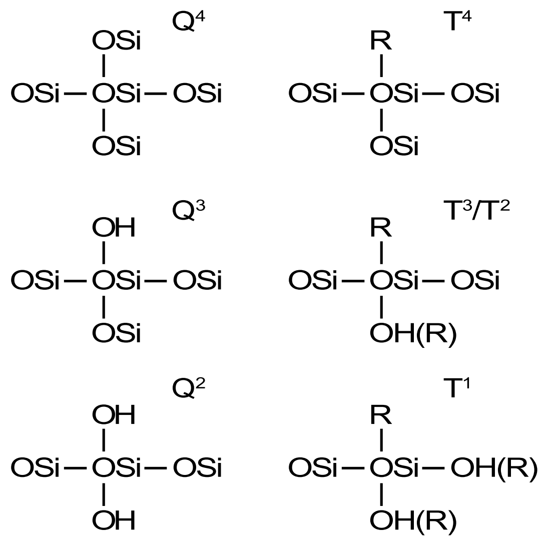


Inspection of equation B suggests that we have a method of quantifying CP spectra (by obtaining S_0). And, indeed, we do - in principle. We can either obtain an array of spectra as a function of contact time and fit the intensities to equation B, to give us S_0, T_{XH} and $T_{1\rho}^{H}$. Or, we can plot $\log_e$(intensity) and find the zero time intercept by projecting the long time behaviour. In both cases, though, it is essential to use integrated intensities so that differences in linewidth do not influence the result and to take into account any spinning sidebands.

To illustrate the analysis of the contact time behaviour we will look again at the result for the modified silicate shown on the previous page. The full array consisted of 13 values. The signal as a function of the contact time for three of the signals is shown in the plot below. The solid lines are the fit to equation B. In each case we a get a reasonable representation of the experimental data. The numerical values generated from the fitting are shown in the table and the notation used to describe the signals is explained on the next page.



Signal	T_{SiH} / ms	$T_{1\rho}^{H}$/ ms	Intensity / %	DP
Q^4	4.7	39	34	38
Q^3	3.0	23	29	26
Q^2	2.4	19	4	4
T^4	1.8	28	14	15
T^3/T^2	1.8	22	17	16
T^1	2.2	20	2	2



There are several observations we can make about this result. The intensity values are close to those that come from a quantitative direct-polarisation experiment (the separate column labelled "DP"). However, there is still a significant difference in Q^3/Q^4 compared to the DP result (1.17 by CP, 1.46 by DP). This is always likely to be biggest source of error in a "quantitative" CP measurement. It is feasible that nuclei very far from a proton do not cross-polarise at all and therefore do not contribute to the spectrum. It is worth noting that the CP experiment took 5 hours and the DP one 16 hours, although for a case where both experiments are feasible the "safe" option is the DP experiment even if it takes longer. The Q^4 environment is also the slowest to cross-polarise (T_{SiH} = 4.7 ms) because it is further from a proton than any other environment. Perhaps, surprisingly, its $T_{1\rho}^{H}$ value is also significantly longer than for any of the other species. Unless the CP signal for the Q^4 environment originates from protons that have no association with other environments (which is hard to believe) this observation is difficult to understand. It suggests that our relatively simple treatment of the CP behaviour may not be properly applicable in this case. Whatever the cause, it illustrates some of the problems that can be encountered with CP experiments used in this way. Of course, we could remove the simplifying assumptions we made to produce equation B and reintroduce $T_{1\rho}^{X}$. That then means we have to fit our data to an extra variable which is probably equally fraught with uncertainty, especially given that we have only 13 data points in this case!

Other things to be aware of in CP experiments

Even if we treat our data with the full equation given earlier, it does not mean we can accurately reproduce the contact time behaviour. For example, there are circumstances when we can observe oscillations in the first part of the curve. This can happen when the "abundant" nucleus is relatively isolated so is only weakly coupled (if at all) to neighbouring like nuclei. Analysing the CP curve in a different way in these circumstances can give accurate information on the H-X (or F-X) separation.

Cross-polarisation relies on the dipolar coupling between the abundant nuclei to make it efficient and it is possible to experience difficulties in obtaining CP spectra in systems where such nuclei are relatively dilute.

The whole treatment of the CP process in this section has worked on the assumption that the CP is from an abundant spin to a low natural abundance X spin. Do not expect the same theory to work for CP between H and F (the theory for that is much more complicated) or for CP between two spins X and Y neither of which is H (or F). In both of these cases the CP experiment tends to be used to establish the relationship between spins or as a filter, rather than for sensitivity enhancement. Nevertheless, optimising the contact is still straightforward - vary it until you get most signal.

Finally, if you get a chance to check the contact time behaviour - make a careful note of it. For example, when working with a pure, nitrogen-containing organic, its usually easy to determine the signal behaviour as a function of contact time for the carbon. Because the proton source for H to C CP is usually effectively the same as that for H to N CP, the behaviour for carbon gives you a good clue as to the contact time to use for ^{15}N - given that the latter signal will probably have too low an intensity to optimise separately. For ^{15}N we find that we get the best spectra when $T_{1\rho}^{H}$ is long (so when there is signal in the carbon spectrum at long contact times).