

Practicum 2, Spring 2005

© Anne-Frances Miller, 2005

Optimizing the sensitivity of a 1d -part 1.

R.B.'s coffee, dried and redissolved in $^2\text{H}_2\text{O}$

Today we will perform a 'smart' 1d experiment. Here the objective will be to gain some understanding of the experimental parameters and make intelligent choices for them with the objective of collecting the most intense and artifact-free spectrum. In the description that follows procedures that have already been detailed are not detailed again, and when you have to click on a 'button' the button's name is outlined, when you have to type a command the command is in **bold** and when I refer to a parameter it will be underlined.

Clean the sample, load the sample, check hardware configuration

Lock (don't saturate), preshim Z1 and Z2, set you spinning speed and confirm temp = 'n'.

Gradient shim. (For fun some day, collect a 1d before and after gradient shimming to see the improvement shimming well makes.)

When you have a set of shims you like you can save these as a shim file with the **svs** command (go to the shims directory first please). Similarly, parameter sets can be saved (in parlib) with **svp**. Alternately, both can be obtained from an older experiment in which they were used by reloading that .fid directory, setting **load = 'y'** so that your current shims will be overwritten and then **su**. The default value for load is 'n', for obvious reasons.

Type **s2pul** to load basic parameters and this basic pulse sequence.

S2pul is one of many macros intrinsic to VNMR that will execute series of VNMR commands and modify parameters. Among other things, this macro (and John Layton's gshmend macro) will reset a couple of hidden parameters which you should therefore be aware of. spin? reports whether the spinner is on or off, but unfortunately does not give you control over it. To turn on/off the spinner you have to open acqi and then lock. The spin speed should be 20 Hz when on and should be off for all 2D and higher experiments (you will find that I tend to work with it off out of habit, whereas 'small molecule' people will tend to automatically have it on, so check the parameters of your experiment instead of assuming that it is still as you left it.) Also check that wshim='n' and alock='n' otherwise when you launch your experiment with a ga or go command the machine will automatically proceed to reestablish lock and reshim, overwriting your own shims.

The name of the pulse sequence to be executed resides in the parameter seqfil (pulse sequence file). Thus, after executing the **s2pul** macro, seqfil='s2pul'. The s2pul (or std1h) sequence encodes the most basic, and frequently used 1 pulse sequence consists of an excitation pulse, a period during which an FID is digitized and then a delay during which magnetization is allowed to recover (relax) before the sequence is repeated. Even this very simple sequence of events encompasses a number of decisions (adjustable parameters) that can affect the quality of the spectrum that results.

pw: pulse tip angle A 90 degree pulse will produce the largest signal in a single scan, but not necessarily the most signal for a fixed amount of time, when a pulse sequence is repeated many times. Nonetheless, it is good to know what tip angle is being used, or equivalently the duration of a pulse effecting a 90 degree tip. This value depends on the power (tpwr) used and is typically stored as the value of pw90. This value is best determined by measuring the pw360 and dividing by four (guess why). Note that most pulse sequences do not actually use pw90, it is just stored in the experimental parameter set as a convenience to you.

tpwr embodies the power used during the pulse (the size of the H_1 magnetic field tipping the magnetization). Large values signify high powers, but the units used are those of attenuation: db. The decibel scale is logarithmic. a 1 db decrease in tpwr will decrease the size of H_1 by 12% and thus require that pw be 12% longer to achieve the same tip angle. The easiest relation to remember is that a 6 db decrease in tpwr can be compensated for by a 2-fold increase in pw. The preceding refers to ideal probe behaviour, or linear behaviour. However, at the high end of the power scale, the probe's power handling capability is less efficient and eventually saturates. This is called the compression regime and it means that you have to push harder and harder to shorten your pw90 at high powers. It also indicates that you are approaching the probe's limits (yellow light). For this reason, we often check probe linearity before proceeding with an aggressive experiment. If you ever encounter probe arcing (below), stop whatever you are doing. This is the red light. tpwr can take integer values between -16 and 63, but again, don't use top power unless you need it AND it is not stressing the probe.

Finding the pw90:

Choose a reasonable tpwr, such as **tpwr=57** for ^1H . Set pw to a value you are confident is much smaller than a 180° pulse. **pw=2**. This will set pw to 2 μs , the default unit for pulse widths. Take a second and **dps** before launching the experiment. The different time units are colour-coded, always be sure that your pulses are in μs (or occasionally in ms), NOT s. Note that changes in tpwr will alter the height of the pulses shown in dps. Compare the results of **tpwr=60** and **tpwr=10**. **ga** to launch the experiment and automatically produce the Fourier transformed spectrum. Phase it up.

Now compare the results of using an increasingly long pw. This is accomplished in a single experiment by setting up an array of pw values, trying each of them and displaying the resulting spectra side-by-side for easy comparison. Type **array**, then respond to the prompts with **pw, 10, 5, 5**, for comparison of the effects of 10 pw values beginning at 5 μs and spaced by 5 μs . Before interpreting the results be sure that the **absolute** spectral intensities are displayed (as opposed to internally normalized intensities), by typing **ai**. (Normalized intensities are obtained by typing **nm.**, but don't do that now). **dgai?** gets the machine to tell you whether it is in ai or nm mode.

This should correspond to tipping magnetization further and further around the axis of the H_1 field applied during the pulse (eg. -x). Thus magnetization should appear along y, then shrink along y as it grows along -z, then grow along -y as it shrinks along -z, then shrink a long -y as it grows along z. Once it returns to maximum along z and zero along y it will have executed one complete precession of 360° and the length of pw required to accomplish this is the pw360. (Note in the preceding that the only magnetization we detect is the $\pm y$ magnetization.) Calculate the pw90 from the pw360/4.

If your first array did not include the pw360, create a new array with longer pw values and possibly smaller spacing for a more accurate pw90. If you are checking for probe linearity, determine pw90 again for a tpwr 6 db smaller than the one you just used. It should be twice as long. Write your results in the log book, to help John Layton keep track of spectrometer and probe performance (thank you).

In cases such a pulse width calibration when the results of a series of scans are to be compared, it is useful to be able to display all the spectra simultaneously. **dss** will overlay them all. **dssa** will stack them one above the other vertically, with the first member of the array at the bottom. **dssh** is what we used, to display stacked spectra in a horizontal row with the first spectrum on the left (display stacked spectra horizontally). To plot the whole stack of spectra use the pl command with the option all: **pl('all')**. To view the array of values which your arrayed parameter takes, type **da**.

If you ever see weird or random behaviour in response to a smoothly increasing pw, this may be probe arcing. IMMEDIATELY abort the experiment by typing **aa**. You can also use the abort acquisition button or type **stop**.

Assuming the probe is linear, enter the higher value of tpwr and the corresponding pw90 as pw in your experiment.

Spectral width

Correct identification of solvent is necessary for tof to be meaningful and reproducible. Make sure this is set correctly before proceeding.

sw: spectral width (or sweep width in the old days of CW spectroscopy) This is the width of the frequency domain spectrum that is to be obtained after Fourier transformation of the data. Since spins with a frequency very different from the carrier frequency precess very rapidly in the rotating frame, data points must be collected very rapidly in order to determine their frequency. The Nyquist condition defines the relation between the "dwell time" between acquisition of complex data points (dw) and the maximum frequency (relative to the center of the spectrum, sw/2) that will be properly contained in the spectrum.

$$dw = 1/sw$$

Resonances outside the spectral window will be wrapped in and attenuated by filters.

Begin by collecting an nt=1 spectrum with a wide sw your are certain will contain all your resonances (30 ppm is tons for most purposes). The resulting spectrum will be extra noisy from the noise of all the extra data points collected, but will allow you to chose a spectral window that does not cut anything out. Place cursors around your spectrum, expand and type **movesw**. This will move the tof as well as the sw to give the requested spectral window. tof denotes the center of the spectral window frequency (transmitter offset).

Note that if you have one very strong signal, for example that of residual water, your best bet is to center the spectrum on that signal, even though this may require you to use a slightly larger sw to include all of your resonances. The reason is that if the strong signal is very strong then artifacts associated with it may be sufficiently strong to contaminate your spectrum. In this case, don't adjust sw first. In your wide trial spectrum place the cursor on the one strong signal and type **nl** (nearest line) to have the computer optimize the cursor position to the very top of the line, then type **movetof** to place the tof at that frequency. **ga** to collect another spectrum at the correct tof and place cursors on the farthest-flung line and the strong central line. You will want sw = 2 times this width plus ten percent for baseline correction purposes later. Set **axis='h'**, then **sw=2.2*delta**. The former is necessary because sw is in Hz unless it is entered with a 'p'.

We will look a aliases produced by improper choices of sw and tof. Other macros that will help you choose a sw fast include **minsw**, **sw(down,up)**.

Acquisition time

The duration of the acquisition time determines the maximum possible spectral resolution, as only frequency differences $\Delta\nu > 1/at$ will be resolvable. With a strong sample, you can always start with a long at, and collect 4 or 8 scans. Process without line broadening (later lecture) and evaluate the width of your sharpest lines. This is the limit determined by the quality of your shimming, NMR tube, sample contents . . . There is no point in

digitizing to higher resolution than these afford. For an observed limiting line width at half height $\Delta\nu$, set $\underline{at}=2/\Delta\nu$ for maximum resolution. This provides for the possibility of resolution enhancement (later).

If you cut \underline{at} too short, you can truncate the fid and produce sinc wiggles called truncation artefacts (which we will explain in lecture). These wiggles look a bit like feet. Weighting functions were devised to shape the fid, squeezing it down to zero at the end *as if* it had fully decayed. Subsequent Fourier transformation produces broader but normal-looking signals (without feet) so application of the weighting function is called apodization.

d1: relaxation delay

When insufficient time is allowed for relaxation each successive scan produces less signal, the phase cycle fails to cancel artifacts effectively and signals that relax slowly are preferentially suppressed. We will deal with this in more detail next time. For small molecules, allow several seconds. Optimal sensitivity after a 90° pulse is obtained when $\underline{at} + \underline{d1} = 1.3 * T_1$. $\underline{at} + \underline{d1} = 3.7$ is a good choice. The default units for $\underline{d1}$ (and all delays) is seconds (s). Set $\underline{d1}$ to some value and then in dps, check the colour coding of the delay.

Others

gain, make sure that you make good use of the spectrometer's dynamic range by using a gain of 30 or higher whenever possible (*i.e.* without having the receiver overload light come on). You can have the spectrometer choose a good value of gain by setting **gain= 0 gain='n'** before acquiring the spectrum. The spectrometer will then collect single shots of your spectrum with a series of different, increasing gains until it starts to approach the capacity of the receiver. You can then learn what it chose using **gain?** or take back control of gain without changing it by typing **gain='y'**.

If your gain is set too high, you can damage the receiver by subjecting it to too much power. You can also damage your spectra. When the amplitude of the incoming fid exceeds the dynamic range of the receiver, the highest possible value is recorded but this will be less than the actual value the amplitude should have had. The fid will have its top 'clipped' off. Fourier transformation of this flat-topped fid produces sinc wiggles, but due to their different origin they cannot be apodized away. You have to decrease gain and collect your data all over again. Better to save time (and the receiver) by optimizing the gain first on single shots before launching a long run. NOTE that certain 2Ds like COSY will produce small signals in early increments and much stronger signal in later increments, so the gain should be optimized on a scan set up to look like a mid-run increment.

Choice of nt, bs affect the effectiveness of the phase cycle, and whether or not data are stored in the event of premature termination of the experiment. For long experiments, set bs = some multiple of 8 that consumes ≈ 5 minutes. Then you will never lose more than 5 min. of data, and you will be able to see updates in the spectrum after reasonable (significant) numbers of scans have been added to it. Set nt to some integer multiple of the phase cycle, which is commonly 8 for the experiments we will be doing at first. eg. **bs=8 nt=16**.

Be sure dp = 'y' for double precision in digitizing your data (this is the default).

For a truly gourmet baseline, and before collecting 2d spectra:

Set tof and sw, and rephase, then optimize the gating delays rof2 and alfa

rof2 is allowed for the power delivered in the pulse to decay to zero before the (very sensitive) receiver is turned on. alfa is allowed to allow the amplifiers involved in signal detection to stabilize before the first data point is collected. When dsp is set to 'r' (digital signal processing in real time) data points are collected at very close intervals, and errors in the synchronization of the pulse timetable and that of the receiver produce baseline roll and first-order phase errors. First get the computer to reset rof2 based on your current phases by typing **crof2**. Then optimize alfa keeping the sum of the two constant and arraying alfa. This pair of values should

then be applicable to essentially all experiments. The commands to use to find an optimum pair of rof2 and alfa values are: **array='(rof2,alfa)'**, **rof2= x, y, z**, **alfa= a, b, c**. Where a,b,c,x,y,z are in μs and $a+x = b+y = c+z =$ the sum of the rof2 and alfa that were obtained upon typing **crof2**

Collect a gourmet spectrum (**dps** first). Save your parameter set.

Show an example of spinning side bands, especially when XY shims are poor.

Distinguish from ^{13}C satellites.

Reading for next week:

Chapter 3.1 and 3.2 (3.4.4 optional) 3.5.1 pages 94- 97 (top 2/3) from "High Resolution NMR Techniques in Organic Chemistry" .