

Practicum 2, Fall 2010

Optimizing the sensitivity of a 1d -part 1.

Strychnine, dissolved CDCl₃

Today we will get 'smart' about how we perform our 1d experiment. The objective will be to begin gaining some understanding of the experimental parameters so that we will be able to make intelligent choices and collect the most intense and artifact-free spectra. In the description that follows procedures that have already been detailed in Practicum 1 are often not detailed again.

Clean the sample, load the sample, lock and shim (see practicum 1).

Open experiments > standard 1D experiments > proton

This experiment (and all the other pre-packaged experiments) come loaded with some hidden parameters of which you should be aware. We are not prepared to deal with all of them yet, but we will begin introducing some of them today. These experiments will generally function well (remarkably well), but for optimal performance all of them will need some tweaking.

Even this very simple sequence of events encompasses a number of decisions (adjustable parameters) that can affect the quality of the spectrum that results. Today I want to look at the duration of this pulse, which is known as the 'observe pulse' or the 'read pulse'. Like all pulses, it causes magnetization to rotate around the axis along which the pulse's magnetic field is applied. What this means for us is that magnetization which is aligned with the field of the magnet (along Z) will rotate around the X axis of this pulse, and this rotation will bring the magnetization into the X-Y plane where we can detect it. If the pulse is along X magnetization will rotate in the YZ plane, and a 90° rotation will bring it into alignment with Y. A 180° rotation will cause it to invert and point along -Z, whereas a 270° rotation will bring it to -Y.

Thus, a 90° 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). We would like to be able to use a 90° tip angle, and to be able to choose different angles if we prefer (eg. for slowly-relaxing spins).

The higher the power used for a pulse, the shorter the time needed to tip magnetization by 90°. Therefore when we determine the 90° pulse length we will need to know the power to which this value applies. The VNMR names for these values are pw90 and tpwr, where pw means pulse width and is whatever duration of pulse you may happen to be using, which pw90 is the specific value that tips magnetization by 90° at the power 'tpwr' (transmitter power).

pw90 is best determined by measuring the pw360 and dividing by four. This practice eliminates errors that can arise due to slow relaxation (later).

tpwr has the units of db. The decibel scale is logarithmic. A 1 db decrease in tpwr will decrease the applied field 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. For this reason, we often check probe linearity before proceeding with an aggressive experiment. You should not do this unless it is necessary, and you should speak with Mr. Layton or Prof. Miller FIRST.

Finding the pw90:

Acquire>Acquisition ([1] in **Figure 1**). Begin by collecting a decent ¹H 1d. Use a observe pulse that you are confident is less than 90 °. Starting value of 2 will never be wrong [2] (although your spectrum might be weak). Entering the pulse width into this box will ensure that it is used with the units of microseconds. This is the default unit for pulse widths. You can check on that by clicking Sequence [3]. In the pulse sequence display the length should appear above the pulse in blue lettering [4]. Microseconds are provided in blue, milliseconds in orange and seconds in white, by convention [5].

Choose a reasonable power, 62 is typical [6]. (Note that the screen says that this is the calibration power, but it is in fact the current power that will be used for the experiment.) The lowest allowed value is -13 and the highest is 63.

You can check on this too because changes in the power will alter the height of the pulses shown in the pulse sequence display. Compare the results when the Power is entered as 0 vs. the results when it is entered as 62 [7].

Click on Show Time [8] to confirm that you will get what you think then Acquire [9] to launch the experiment and automatically perform whatever processing is prescribed in 'Future Actions' [10].

The spectrum you obtained will have been phased up, but the spectra we anticipate from our arrayed experiment will have different phases and these are significant. As a precaution we would like to de-activate automatic phasing and replace it with use of a single set of phase values for every single spectrum in our planned array. Go to 'Future Actions' and replace 'process' with 'wft dc'.

Choose a number of scans that will give decent signal to noise in the minimal amount of time [11]. Set 'Steady-State' scans to 0 or turn them off [12].

Now we will prepare to repeat this same experiment varying the pulse width. In VnmrJ the set-up is done by clicking on 'Arrays' [13].

A new window opens (Figure 3, [1]). In it, enter the name of the parameter to be arrayed ('pw') and return [2]. The rest of the line should then populate and the

entry blanks below will contain the current value(s) [4]. Go to the entry blanks below and enter the number of values you wish to compare [3], the first value [4], the amount you'd like added to that value each time the experiment is repeated [5], clicking return after each. The last value should self-populate and the table on the right should also populate, giving you a chance to review your choices. Note that we are doing a linear array this time, but in other cases an exponential array will be very useful (later). Also you can start with a high initial value and provide a negative increment. Note however that some parameters are not allowed to take on negative values, or values above some present limit and those limits may truncate your series. Close [7].

The 'Show Time' [8] command button reveals that the array will require 1:32 (1 minute and 32 sec.) to execute.

De-activate autogain for all arrayed. A good value to use is the one that was visible before minus 6 [8]. This is because we used a small tip angle pulse before and so got an artificially small signal.

Acquire [9].

You can monitor the progress of this and other slower experiments by watching the feedback line [1] (**Figure 3**) but to get a more detailed overview of the status of your experiment, click on the small triangle to the line's left [2], to open yet another window [3]. That window can be closed with a second click on the small triangle [2].

When the experiment is complete, you will see the processed last spectrum. To see them all, take advantage of the panel on the left hand side (LHS) of the screen.

Choose the In the LH panel choose the 'Arrayed Spectra' tab (**Figure 4**, [1]).

Under Chart Dimensions, you can alter the Vert. Height [2] of the spectra and their Vert. position [3]. As for shimming and locking, click the LMouse to decrease values, RMouse to increase them, and MMouse to alter the increment size. You can have the individual spectra numbered [4] or associated with the parameter value that produced it [5], based on your selections.

You could also begin by scaling one of the spectra in your set by selecting which spectrum [6] (for spectrum 1 choose start and stop values of 1) or display the first one with 'display 1D' [7]. Then you can use the middle mouse in the graphical display window (the black one) to size and move the spectrum (including zooming in on one area) before showing all the spectra together again by clicking [8] or [9] (horizontal array or vertical stack). (in Process>Display choose 'Absolute' amplitude scaling.)

Figure 5 shows a horizontal array of spectra labelled with the pulse length.

You see that the spectra begin with positive intensity, shrink, invert, grow, shrink again to a second null before beginning to grow back as positive spectra. The second null, when they cross from negative to positive intensity corresponds to a 360 ° tip angle (the first null crossing from positive to negative corresponds to a 180

° tip angle). The second null is the one that is resilient vs. relaxation. Take the pulse width that produced this spectrum and divide that value by 4 to get the duration of pulse that will produce a 90 ° tip angle. You may need to do some interpolating, or to run a second array with a smaller step size focussing on a specific range of values. In the case depicted by **Figure 5**, pw360 is a little larger than 70 us. Using an estimate of 72 us yields a pw90 of 18 us (at a power of 59). (**Figure 6**) **Figure 5** also illustrates yet another way to see the values that produced the data. Selecting Acquire>Overview provides a simple spread-sheet of parameters with their VNMR names and their values. This can be VERY useful. Click on the 'Text' option [3] to get this.

If your first array did not include the pw360, either increase the increment size or start at a longer value of pw.

If you ever see weird or random behavior in response to a smoothly increasing pw, this may be probe arcing. IMMEDIATELY abort the experiment by clicking on **Stop** [4].

To stop collecting arrays of spectra and implement your pw90, either enter that value in Acquire>Acquisition 'Observe Pulse', (also resetting the values for pw90 and Power as needed). The Observe Pulse value should now be associated with the tip angle 90 ° (**Figure 7**).

If you are checking for probe linearity, determine pw90 again for a power that is 6 db smaller than the one you just used. The new pw90 should be twice as long, if this is true then the higher power is safe to use. If the new pw90 is less than twice as long, then the higher power was too high.

If you got a lower-power pw90 that was less than twice the high-power pw90, decrease the power further, re-measure pw90 and find a region of powers in which the probe is linear. Work only in that region.

Gain

You would like to be at a large gain that nonetheless does not saturate the receiver. Above gain=30, higher gains do not help. To have the instrument choose a good gain, use autogain in Acquire> Acquisition (**Figure 8**, [1]). The spectrometer will then collect single shots of your spectrum with a series of different gains until it starts to approach the capacity of the receiver.

In general, collecting with higher gain yields a larger signal (**Figure 9**). However the signal-to-noise ceases to increase above gains of ≈ 30 . This pair of spectra were collected with gains of 30 (left) and 44 (right), respectively. They are displayed side by side by selecting Show (•) spectra Horizontally [2] in Process>Display. They are displayed with 'Absolute' intensity [1] (no scaling) in Figure 9. However if they are displayed each normalized to the largest internal peak ([1] **Figure 10**), you can see how much worse the high-gain baseline is. Furthermore, when the amplitude of the incoming data 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 free induction decay (FID) will have its top 'clipped' off. (**Figure 11**). Fourier transformation of this flat-topped FID produces sinc wiggles. The clipping is far from severe, it is actually barely obvious, but it already affects the quality of the spectrum.

You can also damage the receiver if your gain is too high, by subjecting the receiver to too much power. If you mis-set your gain, you will have to correct it 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 if you are running in proteated solvent, such as 90% H₂O, take your first spectrum with gain = 0, to be conservative. Also, 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.

Offset

Last time we dealt with this by collecting an extra wide spectrum putting cursors around it, zooming in and then clicking on 'Move SW'. That action changes both the spectral width (sw in VNMR) and the centre point of the window to be observed (called the transmitter offset, the offset, or 'tof'). The tof was whatever value happened to be the centre of the window you chose. However 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. 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. Display the axis in Hz and view the splitting between the cursors (right-hand number in the display window). Use double that value for the sw (Figure to follow).

Later we will look at 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 (at) determines the maximum possible spectral resolution, as only frequency differences $\Delta\nu > 1/\text{at}$ will be resolvable. With a strong sample, you can always start with a long at (eg. 3.5 sec), 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 etc. . . There is no point in digitizing to higher resolution that

these afford. For an observed limiting resolution (closest peaks you can distinguish or sharpest line width at half height) of $\Delta\nu$, set $\underline{at}=2/\Delta\nu$ for maximum resolution. This allows 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 artifacts (which we will explain in lecture). These wiggles look a bit like feet. Next week we will experiment with weighting functions that 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 the acquisition time plus the relaxation delay ($d1$) are $\underline{at} + \underline{d1} = 1.3 \cdot T_1$. We will discuss T_1 (and T_2) soon. $\underline{at} + \underline{d1} = 3.7$ is a good choice.











