

Practicum 3, Spring 2005

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Optimizing the sensitivity of a 1d -part 2. (ethyl crotonate in CDCl₃)

IMPORTANT: know the ways to stop a run immediately, and the times when you should do so. How to: enter **stop**, enter **aa**. Click on the **abort acquisitions** button under main menu.

When to: whenever the receiver overflow light is on. Whenever you even suspect that a pulse is too long or a power is too high, if the machine loses lock, the temperature goes off, you hear anything unusual smell anything suspicious etc. etc etc. You can always restart a machine that is not damaged. However, once damage is done, it can be weeks before the machine can be used again. Whenever you set up long runs in a queue, make sure you launch each one and let it run into the sequence proper (past the ss scans) to be sure you are not overloading anything. Then stop and set up your queue.

TODAY we will perform a quantitative 1d experiment. The objective will be to collect a spectrum in which the integrated areas of the individual resonances are really proportional to the proton concentrations responsible. This involves taking into account the different relaxation rates of the different hydrogen atoms in a molecule. Knowledge of these relaxation rates also enables one to obtain the highest signal-to-noise per unit time, which is valuable for spectra of dilute compounds or rare nuclei (¹³C, ¹⁵N).

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.

Upon starting vnmr: type **explib** to list the text and pulse sequences of all your experiments. This will allow you to choose an experiment which is just what you want, or one you are willing to overwrite. The **text** command and **atext** provide for entering text, 'ctext' clears the text. 'seqfil' is the name of the parameter that is set to the name of the pulse sequence to be used.

Set up an experiment using the pulse sequence name macro: **s2pul**. Disable wshim and alock. Load a clean sample, lock, shim, calibrate the pw at tpwr=57, choose the gain, sw and at. Collect a decent 1d.

Recall from last time, and your problem set, that phase cycling with nt = 4 or 8 eliminates centerband glitch and quad images. Collecting with an adequate sw prevents resonances from 'folding in'.

Common phase notation describes a pulse along the x axis as having a phase of 0, a y pulse has phase = 1, a -x has 2, a -y has a phase of 3. The phase notation applied to the receiver refers to the phase a simple 90° pulse would have had to produce the observed magnetization: although an x pulse (0) produces -y (3) magnetization, the receiver phase is defined as 0.

Measurement of the relaxation times T₂ and T₁.

T₁ and T₂ are the 'characteristic times' or longitudinal (parallel to z) and transverse (perpendicular to z) relaxation. Mathematically, they are the times required for 1/e of the spins of a given type to return to their equilibrium distribution. Just as all systems spontaneously return to equilibrium with their environment ('thermal bath' in physics-speak), the magnetization we placed in the X-Y plane with our pulse will eventually return to the Z axis. This involves two phenomena: the magnetization disappears from the XY plane *and* it reappears along the Z axis, parallel to the static field. Recall that we are considering NET magnetization. Just as hot coffee cools rapidly at first and then increasingly slowly as its temperature approaches that of the room (its bath), recovery of Z magnetization is rapid at first but only reaches 99% complete recovery after a surprisingly long relaxation time. The recovery is exponential (see your book). Of course the magnetization restored along

Z came from somewhere: the XY plane. However, the NET magnetization in XY can disappear faster than it reappears along Z. This is because although the total number of nuclear magnetic moments in the sample is unchanged, they are to some extent aligned together right after the pulse, so they produce a NET moment. After the pulse they precess freely in the static field, so if some spins are subject to a stronger static field than others they will get out of phase with the others and eventually cancel the others to some extent. Thus, NET magnetization decreases by additional mechanisms than those that mediate T_1 relaxation (recovery along Z). Similarly, the spins of protons that 'change identity' via chemical exchange will alternate between different precession frequencies and tend to cancel out faster than the spins whose identities are fixed. The above explain why poor shimming (a non-uniform static field) and molecular and chemical dynamics cause short T_2 values. In lecture, we will give a mathematical proof that short T_2 values are associated with large line widths. The apparent T_2 including the effects of poor shims is called T_2^* while the use of ' T_2 ' is correctly reserved for the intrinsic T_2 that is a property of the molecule and its spins only.

Now for the practical aspects: to choose good values for p_w and d_1 , you should know T_1 , and to choose a_t you should know T_2 . These relaxation times will be even more important in the intelligent choice of parameters used in setting up 2-dimensional experiments (later).

To measure T_1 , obtain a nice 1D, calibrate the p_w90 , type **dot1** (do T_1), you will be prompted for guesses of the T_1 s and the amount of time you are willing to spend measuring them. You are free to overwrite these with your own choice of six or more values: **d2=a, b, c, d, e, f**. Also note the **nt** value the software suggests, and change it to what you want. A 180° p_1 pulse is used to invert all magnetization and followed by a delay of d_2 . By monitoring the recovery of magnetization as a function of d_2 , using a 90° pulse you can fit the recovery profile and obtain T_1 , for different signals. Work with the last spectrum (which should be fully upright). If you had eight spectra type **ds(8)**. Phase it up and set **th** and peak pick using **dpf** or **dll**. **fp(x,y,z)** to tabulate the heights of resonances x,y,z in the peak list. **t1s** calculates T_1 s and **expl** displays the fits (**pexpl** plots them).

To measure T_2 , use **cpmgt2** (invented by Carr, Purcell, Meiboom and Gill for the measurement of T_2). You must know the 90 pulse length (set p_w90). Using $p_w = p_w90$, $p_1 = 2 * p_w90$ and a d_1 of at least $3 * T_1$. In order to measure *bona-fide* transverse relaxation, not just static field inhomogeneity, we will refocus magnetization at intervals of d_2 during the total relaxation interval of bt . set **d2 = .01** for small molecules, **d2 = .001** for macromolecules, $bt =$ values ranging from $4 * d_2$ to $\approx 2 * T_2$ (your best guess, eg. from line widths, in seconds). Display the first spectrum, set **th**, execute **dll** and choose lines for analysis: **fp(x,y,z)**. **t2s**, **expl** and **pexpl** function as above. Note that in addition to T_2 relaxation, 1H spins are subject to J coupling with other 1H spins during the bt interval. This will cause lines to cycle through antiphase and in-phase forms as they decay, and it can be a bit tricky to separate the two effects. For decent estimates of T_1 and T_2 , note that T_1 or T_2 can be estimated from the time needed for a signal to relax half the way, $t_{1/2}$: $T = t_{1/2} / 0.7 = 1.44 * t_{1/2}$ (try to figure out where the factor of 0.7 comes from).

Compare the CPMG T_2 with the apparent T_2 : $T_2^* = 1 / (\pi * \Delta\nu)$ obtained from the line width ($\Delta\nu$, which includes field inhomogeneity, recall that ν is frequency in Hz. Also, for a good line width measurement, use a long a_t , little or no apodization (ie $lb = 'n'$, $gf = 'n'$, $sb = 'n'$). Shim, shim, shim your magnet, shim the lines up tight . . . (to the tune of 'row, row row your boat')

Use of T_1 information

Many molecules' T_1 s are longer than the amount of time we want to commit to $\underline{at} + \underline{d1}$. If you must use a 90° excitation pulse you should allow $(\underline{d1} + \underline{at}) = 1.3 * T_1$ for the longest- T_1 signal in the spectrum (eg. as in all our favourite 2Ds). Otherwise, for maximum sensitivity, you are MUCH BETTER OFF to use a $\underline{pw} \ll \underline{pw90}$. The best tip angle for signals with a given T_1 is called the Ernst angle, α_e , where

$$\cos(\alpha_e) = e^{-\underline{del}/T_1}$$

\underline{del} is the total relaxation time to be allowed ($\underline{d1} + \underline{at}$) and T_1 is the longitudinal relaxation time of the spin of interest (Ernst & Sternlicht, 1972, J. Magn. Reson. 6: 167-182). Using an Ernst angle pulse gets you optimal signal per hour of machine time (when sensitivity matters!). Use the 'ernst' macro. **ernst($T_1, \underline{pw90}$)** or **ernst(T_1)** if $\underline{pw90}$ has been calibrated and the correct value set as $\underline{pw90}$.

If you want to be able to interpret signal integrals in terms of spin concentrations (numbers of identical hydrogens), you must be sure that *all* the hydrogen nuclei are equally relaxed at the beginning of each scan. Therefore you must set $(\underline{at} + \underline{d1}) > 3 * T_1$, at least, where T_1 is the longest T_1 in your molecule. $(\underline{at} + \underline{d1}) > 5 * T_1$ is slightly better.

Use of T_2 information

Weighting functions -1

The signals' line widths reflect their T_2 s: $\Delta\nu = 1/\pi T_2$, in Hz. Broad signal line widths obscure potentially informative couplings, but it is pointless to try to acquire data with a higher resolution than the resonances themselves have. Moreover, if the spectra are noisy, the noise may make it difficult to measure and interpret couplings. Line broadening is applied to the fid (free induction decay) before Fourier transformation, to emphasize the early portion of the fid, in which the signal is strongest compared to the noise. This however has the effect of broadening resonances. For best signal-to-noise apply a 'matched filter' of exponential decay with **lb=x** where $x = \pi \Delta\nu$ and $\Delta\nu$ in Hz is the line width of the resonance in question (the sharpest one, to be conservative).

For a start, **lb = 1/at** is a good conservative choice which will somewhat reduce the noise and not broaden anything more than it is already by the size of the data set. For sine-bell weighting, a first choice might be **sb = 1*at**, **sbs = -1*at**. For Gaussian weighting try **gf = at/2**. For all of these, you can use **wti** to view your weighting function and its effect on the spectrum.

Reading from text: section 2.4