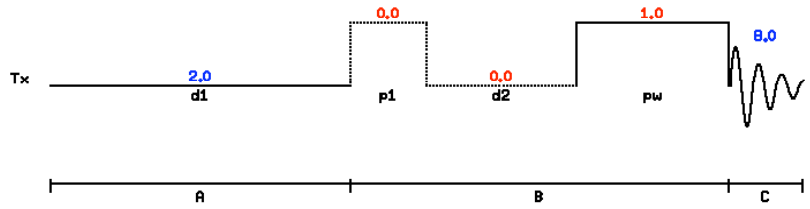
## Kinetics by NMR

The underlying principle behind monitoring a reaction over time by NMR is the ability to repeatedly acquire spectra at controlled time intervals. This is achieved with an arrayed experiment using the pre-acquisition delay (*pad*) as the arrayed variable.

To understand *pad*, consider the following pulse sequence for a 1-D homonuclear experiment:




Ignore the *p1* and *d2* parameters (they are used during an inversion recovery and/or *T1* experiment). The pulse sequence as shown can be displayed in *Varian* with the command *dps* (display pulse sequence). The pulse sequence for one transient therefore consists of (1) *pw* (pulse width), (2) *at* (acquisition time) and (3) *d1* (recycle delay or relaxation time). The number of transients (*nt*) dictates how many of these cycles are run per fid. *pad* in turn introduces a time delay between each fid (see figure 2).

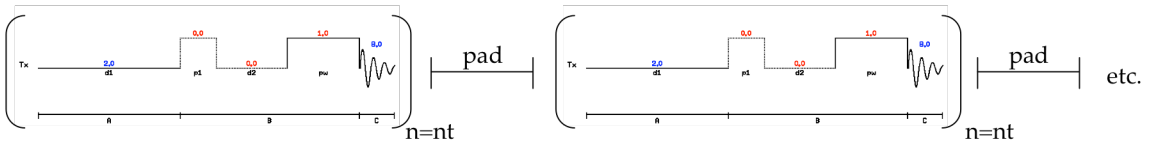



**Figure 2:** Pulse sequence for NMR kinetics experiment.

To set up the array, type *array* followed by *pad*. Enter the starting value for *pad* (in units of seconds). The value may be set to zero so that no time is lost in between spectra. Next, enter an increment size of zero so that the *pad* time does not change from one spectrum to the next. Last, enter the number of steps. This value dictates how many spectra (how many blocks in figure 2) the program should acquire. Do not exceed 1000 or processing will get become difficult.

Once all NMR parameters are chosen, the experiment is started as usual with the command *go*. The command *go* is preferred over *ga* because with spectra being continually updated the analysis becomes quite difficult. With *go* the fid's can be transformed at will and the spectra are not constantly updated.

**NMR kinetics parameters:**

It is imperative to pay close attention to the following NMR parameters. This will maximize the signal and make the integrations quantitative.

(1) Pulse width *pw*:

It is necessary to apply a 90° pulse for a kinetics experiment. This allows one to accurately adjust the *d1* time (reason discussed below). Once the pw(90°) is set, do not change the power (tpwr) because it will affect the value of *pw*. Leave the value of *tpwr* at around 56.

(2) Relaxation delay *d1*:

The repetition time (=d1+at) - the time duration for one cycle - must be sufficiently long to allow the excited nucleus to fully relax before it is excited again with a new pulse. In the case of a 90° pulse, full relaxation can be ensured if $at + d1 > 5*T1$ (90° pulse and *T1* determination are discussed in a separate document). Full relaxation in turn ensures accurate relative integrations.

(3) Offset:

Center the offset on the spectrum by setting the red cursor at the desired location and typing *getoffset:tof.* The pulse will be applied at this frequency and will irradiate the peaks quite evenly. If the pulse were far off center, peaks farther away may *feel* less of a pulse and will result in a smaller relative integration.

(4) Spectral width *sw*:

Set the spectral width to a range that covers all the peaks of interest. Give yourself some room on either side (no need to exaggerate). The spectral width is set in either of two ways: (1) set the cursors left and right of the desired region and type *movesw*. If the range is outside of the current field, type *setsw(max,min)* where *max* and *min* are values in *ppm*.

(4) acquisition time *at*:

The default value will usually be pretty close. By inspecting the Fourier decay (df) one can monitor whether the decay is properly captured during the acquisition time. If the decay is cut short spectral artifact (waves on side of peaks) will appear. In this case increase *at.* If only noise is acquired and the signal has long decayed, *at* may be shortened. The noise can also be dealt with later by truncation in MestreNova (see below).

(5) gain:

The gain is similar to the volume control on a radio. For maximum signal turn it up as high as possible. However, if the gain is set too high there will be an *ADC* error (hissing static noise in radio). In this case turn the gain down.

**Monitoring the Reaction:**

As the experiment is running, the spectra may be Fourier transformed with the command *wft*. This will process as many spectra as have been acquired to this point. The individual spectra may now be displayed with the command *ds('spectrum number')*. With the command *dssh* (display spectra stacked horizontally) all spectra are displayed simultaneously. This is convenient to monitor the evolution of peak over time. One may be more specific with *dssh* by typing *dssh(x, y, z)* where *x, y,* and *z* are the number of the starting spectrum, the final spectrum and the step size, respectively (the step size may be omitted).
To switch from the binned spectral mode back to full screen type *full (*full screen*)*. This command is different from the command *f*, which gives rise to the full spectral width.

The experiment may be aborted early with *aa*. The data is saved as usual with *svf*. Note that the file contains multiple spectra and the size of this file is correspondingly large.

**Processing the Data:**
Import the file to your desktop via *Fugu* and open it in *Mestrenova*. This may require some time. To increase the speed of processing, decrease the line width to 2 or 3. Truncate, backwards linear prediction. Set right and left phase to zero (due to phasing artifact in the *Varian* software).
Caution of window functions. lb will affect all integrations equally but should not be set too high. However, *gaussian*- and *sine*-based window functions can alter relative integrations.