Monitoring Kinetics by NMR

updated: 23 May 2022 (cgf)

Brief Summary

There are two major methods by which a series of experiments can be acquired on a preset time course within TopSpin 3/4. They rely on having good prior knowledge of the chemical/kinetic system and setting up the experiment properly. Compromises may have to be made, the most important being the accuracy of the NMR data versus the kinetic time-resolution; i.e., the time between kinetic measurements will often be dependent on the amount of time needed to obtain sufficiently good signal-to-noise for each experiment.

A. ***Initial Setup:***

1. A good estimate of the kinetic timeframe is necessary for the proper setup of the experiment.

2. See the SampleCase guide for how to switch to manual mode (for persephone and 600):

   http://www.chem.wisc.edu/~cic/nmr/Guides/Ba3vug/AV3_SampleCase-robot.pdf.

   See facility staff to obtain permission to, and for instructions on how to, switch the SampleXpress robot to manual mode on Eos.

3. If needed, see the no-D guide and the solvent suppression guide:

   http://www.chem.wisc.edu/~cic/nmr/Guides/Ba3vug/AV3_noD-NMR.pdf
   http://www.chem.wisc.edu/~cic/nmr/Guides/Ba3vug/AV3_SolventSuppression.pdf

4. Knowledge of relaxation times is critical for obtaining accurate quantitative data. It is important to set **d1** and **aq** correctly (as well as **ns**; see 5 below).

5. Setup the experiment you want to repeat:

   a) Shim on a ***test sample*** that will be as similar to the reaction sample as possible. For example, get the concentration similar so **ns** will be setup correctly.

      Although ECONOMY tubes might be OK, better would be Wilmad 507 or 528 or equivalents. Better tubes will minimize changes in shims — possibly not requiring a shim for optimal resolution — at the beginning of the experiment.

   b) Compromise on **d1** and **ns** only if necessary. Smaller **d1** may compromise quantitation. Smaller **ns** will reduce signal-to-noise (s/n). Kinetic time resolution may be less important than obtaining good quality data.

Discussion on the choice of data acquisition method:

Two methods are available to obtain kinetics data. Method 1 below is easy to perform, and may simplify processing as each kinetic time point is a separate 1D NMR spectrum. This method is, however, less precise as to the timing (start time) of each experiment. Method 2 obtains the data using Bruker's pseudo 2D data format.

→ For slow kinetics — half-life > 20 min — Method 1 may be preferable (Method 2 would even so be at least as good a choice from data quality perspective).

→For faster kinetics, use Method 2.

When processing with MNova, there are no significant differences in workup between the two methods. As a general comment on processing, MNova's ability to perform high-quality deconvolution/peak fitting makes that a good alternative when peak overlap occurs; it may be superior to baseline correction plus integrals. Critical to getting good deconvolution is the quality of the line shape: if it is asymmetric, stay with normal integrals (i.e., in such cases, do not use deconvolution/peak fitting).

B. ***Method 1: Acquiring Separate 1D Spectra:***

1. Acquire a spectrum on the ***test sample*** (see A.5 above) prior to starting the kinetics experiment. Set everything up — **ns ds aq d1** are particularly important — in this spectrum for data quality as you will want during the kinetics run. As mentioned above, sometimes there is a need for compromise between quality of the NMR data and getting data fast enough kinetically. Use your best judgement.

2. Write down the total time to acquire this 1D experiment:

   expt↵ =≈ (**ns**+**ds**)×(**d1**+**aq**)

3. Copy the spectrum and move into that expno:

   wra #↵ then **re #**↵

4. Change the pulse sequence and set the inter-experiment delay:

   d20 = the time per point in the kinetic plot
   e.g., if you want 60 experiments spaced over 3 hr, **d20** = 180/60 = 3 min = 180 sec

   d20 ≥ time written down in step 2 above *(setting this is critical, or TopSpin will crash)*

   ased↵

   PULPROG = kin1d or **kinig301d** or **kinw51d**

   ;**kin1d** is for (the most common) ^{1}H 1D data collection

   ;**kinig301d** is X observe with 1H inverse-gate decoupling (correct for quantitative work), so use this for X{^{1}H} experiments

   ;**kinw51d** includes w5 solvent suppression

   ; all of these pulse sequences are located in /home/topspin/uwchem/pp

5. **ej**↵ ; make sure lift air is on prior to inserting sample

6. You're now ready to start the experiment. Perform the injection, or similar. *Starting a stopwatch is recommended to accurately measure the time between injection to start of the first experiment.*

7. **ij**↵

   lock ; topspin will usually lock automatically, since the solvent is the same

   atma↵ ; not necessary for ^{1}H 1D experiments

   topshim↵ ; perform for slower kinetics

8. **multizg**↵ enter number of experiments to run; will start automatically (see more below)

9. Use **multicmd**↵ to perform commands (such as **efp apk absn**) across multiple spectra.

10. User **.md** to display stack plots. PUBLISH → PRINT is useful. Note the pdf feature there.

11. TopSpin's autoarchiving feature does not work with **multizg** experiments. Use the Linux FOLDERS feature to copy the final data to castor, as shown in Data Archiving to Castor:

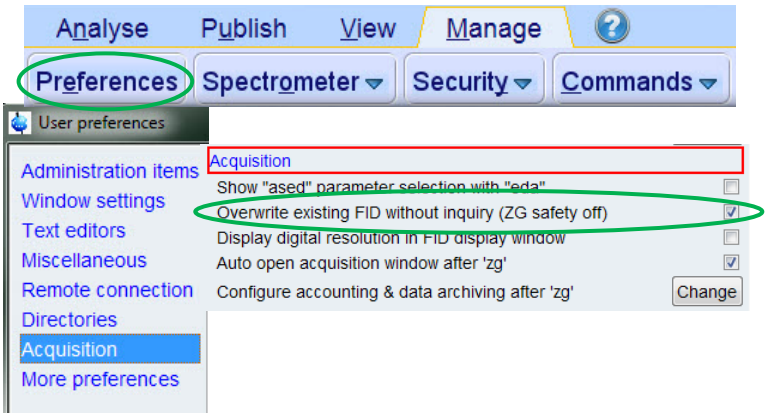
    http://www.chem.wisc.edu/~cic/nmr/Guides/Ba3vug/AV3_TopSpin_Archiving-to-Castor.pdf

Comments on multizg:

1. Suppose **multizg** is started in expno ***i*** :
   a) if ***i*+1, *i*+2, …** do not exist, parameters are copied from ***i*** and run exactly as in ***i***
   b) if parameters already exist in ***i*+1, *i*+2, …** then experiments are run as they are setup; this enables considerable additional flexibility (i.e., could increase **d20** and **ns** for later experiments; could interleave ^{1}H and ^{19}F experiments; etc).

2. Bruker has an au routine **multi_zgvd** that functions similarly, but uses a **vdlist** to control the delay between each experiment, providing more flexibility. See NMR staff for more info.

3. Turn ZG Safety off (by checking the option) in MANAGE → PREFERENCES when using **multizg**.



4. The individual acquisitions cannot be precisely timed; there is a variable delay between acquisitions that depends on the time to write the data to disk. But an accurate start time for each spectrum is contained in the audita.txt file contained in the acquisition folder.

C. ***Method 2: Pseudo-2D method:***

This method is more accurate in timing, and is therefore preferred for fast kinetics.

1. Acquire a spectrum on a test sample just prior to starting the kinetics experiment (see A.5 and B.1 above).

2. Write down the total experiment time for your 1D acquisition using

   expt↵ = (**ns**+**ds**)×(**d1**+**aq**) [usually]

3. Copy the spectrum and move into that expno:

   wra #↵ then **re #**↵

4. Change the pulse sequence:

   ased↵

   PULPROG = kin2d or **kinig302d**

   ; **kin2d** is for standard ^{1}H 1D experiments

   ; **kinig302d** includes ^{1}H invgate decoupling, so use this for X{^{1}H} experiments

   ; these pulse sequences are located in /home/topspin3.1/uwchem/pp

5. Switch the experiment type to 2D, in ACQUPARS A (all parameters); click the  icon.

6. Change the pulse sequence and set the inter-experiment delay:

   d20 = the time per point in the kinetic plot:
   e.g., if you want 60 experiments spaced over 3 hr, **d20** = 180/60 = 3 min = 180 sec:

   d20 ≥ time written down in step 2 above *(this is critical, or TopSpin will crash)*

7. **TD1** will be the number of 1d experiments you want to acquire (60 in the example above), except now they'll be stored as rows in a 2D *ser*ial file (the data are stored in a file called *ser* for 2D data, whereas it is *fid* for 1D data).

8. To simplify processing, set **SI1** = first power of 2 larger than **TD1**: e.g., if you want 60 spectra, then **TD1**=60 and **SI1**=64.

9. To look at a particular row, type: **efp**↵ and enter the row # you want to observe. (**.2d**↵ will return you to the 2D dataset.)

10. To observe the 2D presentation of the data, enter: **xf2**↵ . It may be helpful to turn phase correction on in F2, set **PH_mod = pk** (or set in the PROCPAR panel). Working up the data, at least for most of the processing, will be much simpler in MNova, as it will automatically put the data in stack plot mode.