



Kinetic Profiling by NMR

This note summarises a procedure for obtaining kinetic (time course) data with NMR on **Bruker** instruments using **TOPSPIN**. This provides a means of monitoring reactions as a function of time in the NMR tube and potentially for determining kinetic parameters from the time course data.

1. First tune, shim and run initial spectra of your starting materials **before** the reaction to make sure you know exactly where they appear in the spectrum with the same solvent that you use in the reaction under the same conditions, e.g. temperature, preferably on the same day!
2. Having optimised the spectra of your starting materials alone (tuning the probe and shimming), quickly run the first spectrum of your reaction after mixing noting the time you started (the command **dpa** will display the exact end time of each acquisition and these times can be used in generating time course profiles). If your reaction is not too fast, you may have time to do some quick shimming beforehand; re-tuning is not necessarily required.
3. Having run the first reaction spectrum, increment experiment number (**iexp**), and use the command **multi_zgvd** (*answer the following questions*).

Enter fixed delay (in seconds)

e.g. **600** (10 minutes for spectrometer to wait until next acquisition)

Number of Experiments

e.g **30**

The program should tell you how long your kinetic run should take.

4. Process your first spectrum using **efp** and phase it perfectly, manually. You need all subsequent spectra in the kinetic run to be phased in exactly the same way. Use the **multicmd** command to do this, which asks you the number of experiments required, and the command for processing. Execute with the **efp** command then repeat using the **abs** command (for baseline correction). The macro will use [**efp**] then [**abs**] for all the datasets you have specified; it will phase the specified spectra exactly the same as the first one you phased perfectly.
5. Having now obtained all your experiments, please look closely for your signals of interest, those that grow at the expense of those that decrease. You will then need to integrate them in exactly the same way e.g. integral limits and peak positions, for all *n* experiments.
6. **Integrate** a good example spectrum of the kinetic run, where you have **both** starting material and product and **save** regions to '*intrng*'.

Then write a new integral file with command **wmisc (write new)** with filename xxx, enter a new name.

To be on the safe side, check the integrals are what you want with **rmisc** (read xxx) on another spectrum within your list.

7. When satisfied with your integration of your starting material and product peaks, then go to the first spectrum of the reaction mixture and use the command **multi_integ3** (answer list of questions);

Use EXPNOs(0) or PROCNOs((1)

0

Enter first experiment No

e.g. **2**

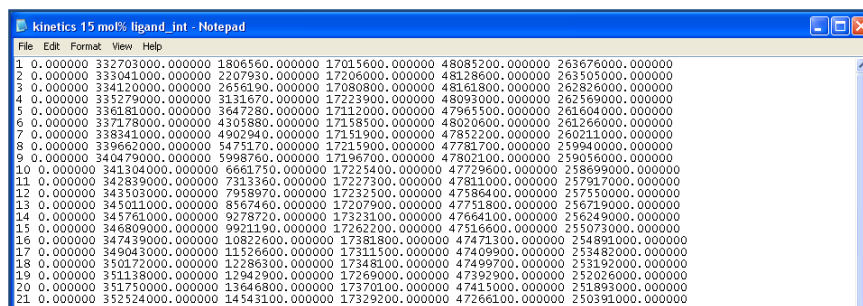
Enter no of experiments

e.g. **30**

Enter name of intrng file**xxx**

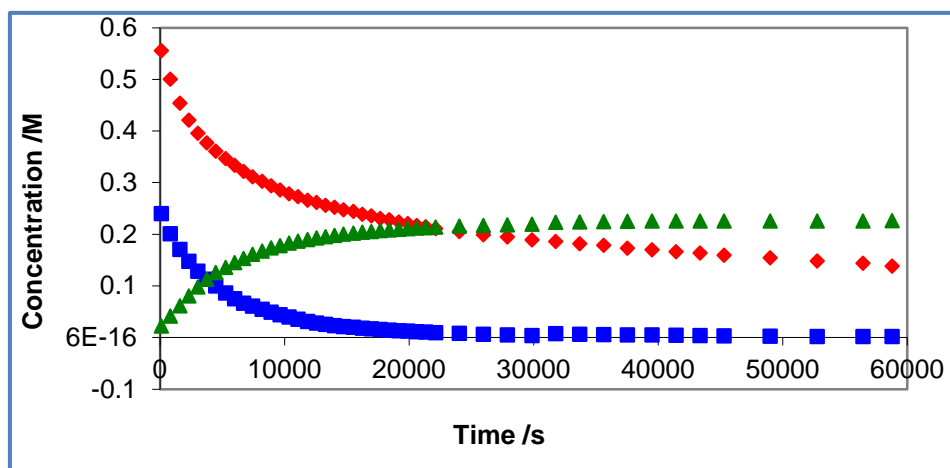
The program calculates all the integrals in files 1.....n and the results are conveniently presented as a txt file in the experiment you performed the **multi_integ3** command in, with the processed spectrum e.g. D:/NMR/data/organic.nmr/file/pdata/1/file_int.txt.

An example of how this text file should appear is shown below;



The screenshot shows a Notepad window titled "kinetics 15 mol% ligand_int - Notepad". The window contains a table of numerical data with 21 columns and 21 rows. The data is organized into columns, with the first column containing integers from 1 to 21. The remaining columns contain floating-point numbers, likely representing integral values for different peaks or components over time.

This table of integral values can easily be imported into **Excel** as a Space and Tab delimited file for plotting and for calculations of rate constants, as shown below.



The in-house au program **readdate** may also be used to generate a text file containing the date and time points for a series of spectra of increasing experiment number (expno) and avoids the need to manually extract the time from each spectrum with the dpa command. The generated file (*kineticdates.txt*) will be stored in the expno of the first experiment. Note: this is not part of the standard Bruker installation and will have to be added to your TOPSPIN installation: copy the au program into the directory:

C:\Bruker\TOPSPIN\exp\stan\nmr\au\src\

(or your corresponding Bruker installation path) and, in your first experiment of the kinetic run, use the command **xau readdate** to compile and run the program.

Please see Tim Claridge or Barbara Odell if you have any problems with these procedures.