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 acqusition)

   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.

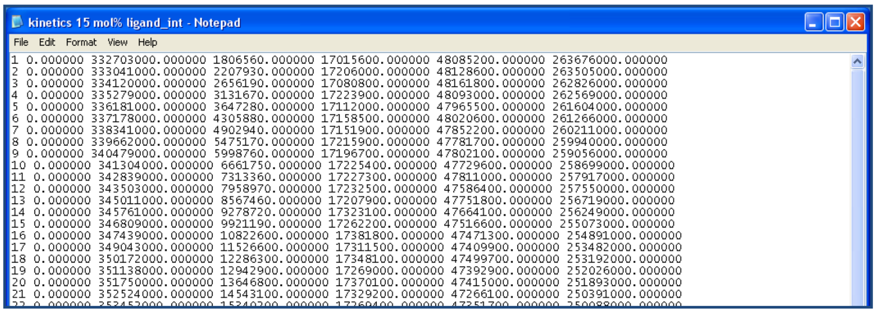
7. When satisfied with your integration of your starting material and product peaks, then go to the first spectrum of the reaction mixture and use the command **multi_integ3** (answer list of questions);

   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

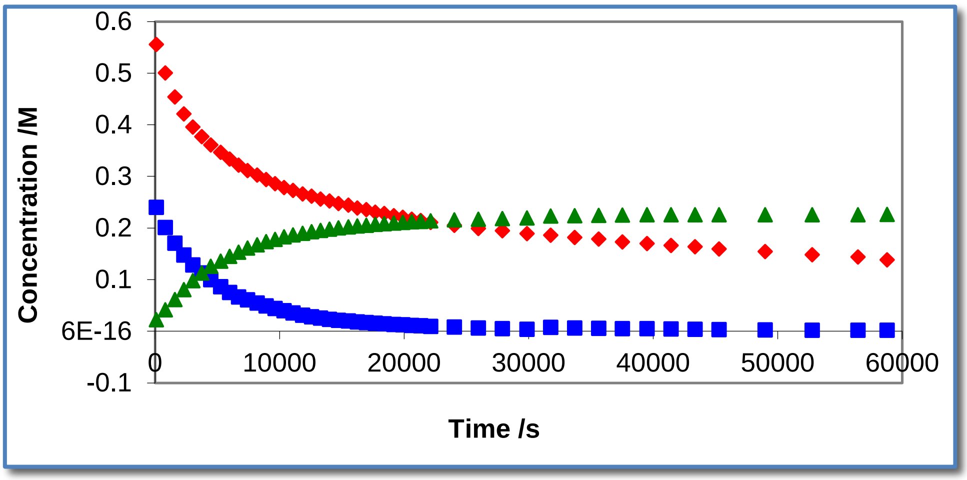
The program calculates all the integrals in files 1…….n and the results are conveniently presented as a txt file in the experiment you performed the **multi_integ3** command in, with the processed spectrum e.g. D:/NMR/data/organic.nmr/file/pdata/1/file_int.txt.

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



```
1 0.000000 332703000.000000 1806560.000000 17015600.000000 48085200.000000 263676000.000000
2 0.000000 333041000.000000 2207930.000000 17206000.000000 48128600.000000 263505000.000000
3 0.000000 334120000.000000 2656190.000000 17080800.000000 48161800.000000 262826000.000000
4 0.000000 335279000.000000 3131670.000000 17223900.000000 48093000.000000 262569000.000000
5 0.000000 336181000.000000 3647280.000000 17112000.000000 47965500.000000 261604000.000000
6 0.000000 337178000.000000 4305880.000000 17158500.000000 48020600.000000 261266000.000000
7 0.000000 338341000.000000 4902940.000000 17151900.000000 47852200.000000 260211000.000000
8 0.000000 339662000.000000 5475170.000000 17215900.000000 47781700.000000 259940000.000000
9 0.000000 340479000.000000 5998760.000000 17196700.000000 47802100.000000 259056000.000000
10 0.000000 341304000.000000 6661750.000000 17225400.000000 47729600.000000 258699000.000000
11 0.000000 342839000.000000 7313360.000000 17227300.000000 47811000.000000 257917000.000000
12 0.000000 343503000.000000 7958970.000000 17232500.000000 47586400.000000 257550000.000000
13 0.000000 345011000.000000 8567460.000000 17207900.000000 47751800.000000 256719000.000000
14 0.000000 345761000.000000 9278720.000000 17323100.000000 47664100.000000 256249000.000000
15 0.000000 346809000.000000 9921190.000000 17262200.000000 47516600.000000 255073000.000000
16 0.000000 347439000.000000 10822600.000000 17381800.000000 47471300.000000 254891000.000000
17 0.000000 349043000.000000 11526600.000000 17311500.000000 47409900.000000 253482000.000000
18 0.000000 350172000.000000 12286300.000000 17348100.000000 47499700.000000 253192000.000000
19 0.000000 351138000.000000 12942900.000000 17269000.000000 47392900.000000 252026000.000000
20 0.000000 351750000.000000 13646800.000000 17370100.000000 47415000.000000 251893000.000000
21 0.000000 352524000.000000 14543100.000000 17329200.000000 47266100.000000 250391000.000000
```

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 Nader Amin if you have any problems with these procedures.