

# SETTING UP WATER SUPPRESSION ON GLENGRANT

## Part 1 – Initial experimental set-up

In setting up the experiment, do as you would normally (see Pete's Guide for Manual Acquisition which you will receive during your hands-on NMR training).

As a reminder, these steps are (actual commands in **bold**):

1. Make new data set

**new**

2. Start lift gas flow

**ej**

*Note: this command is also used to eject sample. Sometimes the gas flow is insufficient to eject sample in which case you should contact the NMR team right away. Personally, I have increased the gas flow (default = 400) to 800 – 2000, this is usually enough to eject the sample. However, this gas flow **needs to be set back to 400 l/min**. which is an optimised value for the sample to be in the right position in the magnet.*

*To change this, go to sample temp. window and change the value under target gas flow.*

3. Place sample in magnet

**ij**

4. Read parameters

**rpar proton.std all**

- 4a. To read NMR parameters for other nuclei:

**rpar**

then read the relevant file in the popup.

For  $^{19}\text{F}$ , read  $^{19}\text{F}$ - $^1\text{H}$  decoupled (or coupled) .std

5. Get all the correct parameters set for this experiment

**getprosol**

6. Read starting shims

**rsh current**

7. Lock solvent.

**lock**

then choose correct solvent

7a. Alternatively, if you know the correct solvent name as used by TopSpin

**lock <solvent>**

8. Set the commands that will keep the lock and lock phase regulated throughout

**autogain**

**autophase**

9. Tune probe

**atma**

10. Set spin

**ro on**

**ro off**

*Note: I've found that setting spin on is good for kinetic studies because it seems to help sample mixing.*

11. Topshim

**topshim ordmax=8 tunea**

*Note: this can take some time, especially if the sample is inhomogeneous. Sometimes I enter 'stop' here when the shim seems reasonable and has stretched beyond 10 minutes, and still have obtained good spectra.*

For non-viscous solvents (i.e. all solvents except DMSO and D<sub>2</sub>O), the NMR team recommend the following, which is faster and better:

**topshim convcomp**

12. Adjust receiver gain

**rga**

13. Zero and go!

**zg**

14. Automatically increase exp no. by 1

**iexpno**

15. Perform multiple zg

**multizg**

## Part 2 – Solvent suppression protocol

1. After preparing the sample with the above, set pulprog to zg (90° instead of 30° pulse)

**pulprog**

**zg**

2. Minimise the receiver gain

**rg 0.25**

3. Perform 1 pulse

**ns 1**

**ds 0**

**zg**

4. Perform phase correction

**apk**

*Note: if the signal is still too strong at this stage, refer to step 4a*

4a. Set P1 to 1us, then repeat

**P1**

**1**

5. Set P1 to 4 x the value given by getprosol during the initial set-up as this will be the accurate, calibrated 90 degree pulse length

**P1**

**4\*the value**

*Note: this is the value for Glengrant but this method is applicable elsewhere using 4 x P1 from getprosol*

6. Adjust P1 slowly and continue until the solvent peak is almost completely suppressed, changing by 1 us each time.

*Usually, it never goes to exactly zero, you get a peak half way to zero, so half is positive, half is negative – but it should look smaller than any positive or negative peak you've seen along the way).*

*Note: this is usually around 54 – 57 on Glengrant for my samples.*

7. Once the peak has been minimized, identify frequency with the lightning tool. Copy this frequency to the clipboard. We want to set this as O1 later. Alternatively, clicking O1 when using the lightning tool should also do the trick.

*Note, with pure H2O, this has to be very exact, which is actually hard to do just by looking... Duncan's first point of call these days is 'findwaterTS3'*

8. Next we read in the presat parameters

**rpar presat\***

(select the **presat.std** file in the pop-up)

9. Set P1 and O1 in acqparms, making sure they are the right values

*Note: on Glengrant, P1 is usually just over 13. O1 is usually approximately 2130 – 2313 Hz.*

10. Do a receiver gain optimization then zg

**rga**

**zg**

*Alternatively, all these steps up to step 9, just before the final rga and zg, can be condensed and simplified by using findwaterTS3*

Type **aunm** (under acqparms, automation tab) then choose **findwaterTS3** and type **xaua**

The screenshot shows the Bruker TopSpin software interface with the ACQPARMS tab selected. The interface is divided into several sections:

- Experiment:** Width, Nucleus, Receiver, Durations, Power, Program, Probe, Lists, Wobble, Lock, Automation (selected), Miscellaneous, User, Routing.
- Automation:**
  - AUNM: findwaterTS3
  - PYNM: (empty)
  - EXP: presat.tro
  - TUBE\_TYPE: (empty)
- Miscellaneous:**
  - CHEMSTR: none
- User parameters:**
  - USERA1: (empty)
  - USERA2: (empty)
  - USERA3: (empty)
  - USERA4: (empty)
  - USERA5: (empty)
  - USERA6: userUSERA5 DEF user acq. parameter 5\$8000
  - USERA7: (empty)
  - USERA8: (empty)
  - USERA9: (empty)
  - USERA10: user
- Routing:**
  - RSEL: (empty)

Or just type **findwaterTS3** at the command line. It optimizes the lock, finds the biggest peak (having O1 nearly correct might help here), then uses a fancy (nutations) method to find the optimal P1.

*Authors Note: I haven't really tried using this much—it's good to always manually set it but may come in handy...DJH Note: ☺ I'd say it's good to know how to do the optimizing manually, in case the*

*automated tool doesn't work...we are a University, so just saying 'push this button' isn't so good from an educational point of view!!*

### **Part 3—acquiring spectra in D<sub>2</sub>O**

After acquiring one set of <sup>1</sup>H data, i.e. see **Part 1**

1. Follow same steps as before

**rpar**

Select 2H.std

**getprosol**

**rsh current**

**lock <solvent>**

**topshim** (see above for how to do this)

2. Initialise the spectrometer—this will turn the lock off.

**ii**

**rga**

**xaua**

*GZH's note: I have not actually acquired a good <sup>2</sup>H spectrum yet with this method, but it should be noted that I was also only experimenting with how to acquire this. Because the spectra were taken in D<sub>2</sub>O, it's possible that the <sup>2</sup>H peak in D<sub>2</sub>O was obscuring other peaks that might have been present.*

## Part 4 – Other useful commands

**tr** transfers dataset so we can have a look at the data before all scans are complete

**apks** automatically phase correct the spectrum

**apbk** fancy way of doing phase and baseline correction in the latest TopSpin versions

**efp** an amalgamation of em, ft and pk to apply line broadening function, fourier transform the data and then apply the current values of zero and first order phase correction

**rg** this reports the current receiver gain and allows you to change the value

**ds** to set delay (or dummy) scans

**ns** this reports the number of scans and allows you to change the value

**stop** usually used to simply stop everything without saving (handy if it all goes wrong)

**halt** to halt the experiment at a certain point, sometimes a defined point i.e. **halt 32**

**Topshim convcomp selwid=1.0** Ensures that correct peak identification in deuterated solvent mixtures; selwid value determines range of ppm for which Glengrant searches