SOP for Pulsed Field Gradient Spin Echo Experiment Used for Determination of the Diffusion Coefficient.

The following instruction assumes that you are familiar with basic NMR data acquisition and processing, using JEOL Delta 4.3.6 software.

1. **90° pulse calibration**
   1.1 **Experiment → service → obs90_check.ex2**
   1.1.1 Load the sample, regulate temperature if needed. Run gradient shim and autolock. Then, set up `obs90_check.ex2` with `autogain` and `force tune` selected. Use enough scans for good signal to noise ratio and submit the experiment. Remember to note receiver gain value reported in the Master Console.
   1.1.2 Copy the position of the peak of interest using *Copy position of the nearest peak to buffer* tool from the *Pick* menu.

1.2 **Experiment → service → obs90_array.ex2**
   1.2.1 *Acquisition tab: x-offset:* double-click the right mouse button to clear the default offset value and click the middle mouse button to paste the offset value copied in step 1.1.2. Then, enter number of scans used in step 1.1.1.
   1.2.2 *Instrument tab:* Enter gain value found in step 1.1.1.
   1.2.3 *Pulse tab:* i. `relaxation_delay:` change 3 s to 10 s (you may need longer delay if $T_1 > 2$ s) ii. Click on `x_pulse` → change start 2 μs to 6 μs stop 50 μs to 60 μs step 2 μs

1.3 Submit the job

1.4 **Analyzing the Data**
   When the acquisition is over, the data will pop up in the *nD Processor* window:
   i. Click on the *1D slice* button. Peak of interest must be phased up: use P0 and P1 to phase it and then click on *apply* and close the window.
   ii. Click on *process file and put in data slate*, press #1 on keyboard (peaks will look like 2D contour plots)
   iii. click on the *select peak* button, then click on the signal you are monitoring. A circle with a cross will show the exact place you need to *pick*.
   iv. After picking, a sine curve will show up (**Fig.1**). You need to zoom in on the negative phase gets to zero. That will be 360 ° array and when you divide that by 4 you will have the 90 ° array of your sample in the solution. (e.g.: $\frac{51.65 \mu s}{4} = 12.91 \mu s$). If you didn’t reach 360 °, modify the array settings and rerun the experiment.
2. **T1 Measurement**

2.1 Experiment → relaxation → double_pulse.ex2

2.1.1 *Header tab* → put the name of your sample

2.1.2 *Instrument tab* → Adjust *recvr_gain* based on the result from 1.1.1.

2.1.3 *Acquisition tab* →

   i. *x_offset* (put the same value you have used for 90° array experiment)

   ii. *scans* → use the same number as in 1.1.1. If the final curve was not clear you can increase the number of scans.

2.1.4 Pulse →

   i. *x_pulse* → use the number you have determined in the 90° pulse calibration

   ii. *relaxation_delay* → change 7[s] to 10[s] or higher (it should be 5 * expected T1)

   iii. *tau_interval* → deselect *list* box

   iv. Change *linear* option to *exponential* as in Fig. 2 → 

   

   

      start: 10 [s] → 10 [ms]

      stop: 10 [s] → 5 [s]

      points: 20

   

   The start and stop settings depend on the expected value of T1. You may need to modify them after first run and acquire the array again.

2.1.5. Submit the job
2.2 $T_1$ Data Analysis

2.2.1 When experiment is over data will pop up in an nD Processor:

i. Click on 1D slice – 1D processor will open with the first slice of the data set.

ii. Peak of interest must be phased down (upside down), correct the phase using P0 and P1.

iii. Apply and close window

iv. Click on data slate icon.

2.2.2 On Delta Master Console window: Viewers → Analysis → Curve analysis (new window opens)

i. Click on the finger icon

ii. Click on File → Open → From finger

iii. Click on your $T_1$ experiment spectrum on data slate (from section 2.2.1)

iv. The spectrum (phased down) will show up on the curve analysis window

v. Locate the position of your peak and pick it

vi. On Mode tab → select Nonlinear Inversion Recovery → Click on Apply

The software will run a curve fitting and report the $T_1$ in the same window (Fig.3).
3. One-shot DOSY NMR

3.1 Low gradient strength and high gradient strength experiments:

3.1.1 First you need to:
   i. turn off the spinning from Sample window
   ii. Increase the lock gain from the Sample window (lock signal should be close to 1000)

3.2 Low Gradient Experiment (Go to Experiment → oneshot_dosy.ex2)

3.2.1 Header tab → Change the name to “low gradient”
3.2.2 Instrument tab → Adjust the recvr_gain to the value used for T₁ measurement and 90° calibration
3.2.3 Acquisition tab → i. x_offset → insert the peak position you have used for T₁ and 90° calibration
   ii. Scans → change 16 to 8
3.2.4 Pulse tab → i. x_pulse → use the number you have determined in the 90° calibration
   ii. relaxation delay → it should be T₁*5
   iii. grad_1_amp → 30 [mT/m]
   iv. diffusion_time → 0.1 [s] – Fig. 4

3.2.5 Submit the job

- When you have collected your spectrum, correct the phase (like the normal ¹H NMR spectrum) and then transfer the spectrum to the data slate.

3.3 High Gradient Experiment (the same Experiment Tool window)

3.3.1 Header tab → Change the name to “high gradient”
3.3.2 Instrument tab → recvr_gain → No need to be changed
3.3.3 *Acquisition tab* → keep all parameters as they were for low gradient experiment

3.3.4 *Pulse tab* → \( \text{grad}_1 \text{ amp} \) → change 30 [mT/m] to 250 [mT/m] or 0.25 [T/m] – **Fig. 5**

![Fig. 5](image)

3.3.5 Submit the job

3.4 *Now you need to overlay your high gradient spectrum on the low gradient spectrum data slate – Determining if the diffusion time is sufficient.*

3.4.1 In the 1D-Processor window click on the *Put Processed Data into Data Slate* button. Data Slate window opens. Now, in this window, click on [ ] and then on the *Open Data as Overlay* button. Next, click on the “low gradient” spectrum displayed in the 1D-Processor. Both spectra will be displayed in the same Data Slate window.

3.4.2 Compare the intensity of the peaks from low and high gradient. If your “high gradient” peak intensity is about 10% (or less) of the “low gradient” peak intensity you can move on and run DOSY experiment. If the amplitude of the 1D data set collected with 0.25[T/m] is higher than 10% of the 1D data set collected with 30[mT/m], re-run the data set with the \( \text{grad}_1 \text{ amp} \) at 0.25[T/m] with a longer \( \text{diffusion time} \), e.g., 0.2[s].

3.5 *One-shot DOSY Experiment* (the same Experiment Tool Window)

3.5.1 *Header tab* → change the title to dosy…

3.5.2 *Instrument tab* → No need to be changed

3.5.3 *Acquisition tab* → scans → change 8 to 16

3.5.4 *Pulse tab* → i. \( \text{diffusion time} \) → as determined in 3.4.2

ii. \( \text{grad}_1 \text{ amp} \) → deselect List, check linear box

start → 30 [mT/m]
stop → 250 [mT/m] or 0.25 [T/m]
steps → 11 [mT/m] (it will be 20 data points)

3.5.5 Submit the job

3.6 DOSY Data Analysis

3.6.1 When Experiment is over data will pop up, click on the 1D slice:

i. Correct the phase (similar to 1H NMR), Apply and close the window
ii. Click on data slate
iii. On Delta instrument window select Viewers → Analysis → Curve Analysis
iv. Curve Analysis Window → Click on finger → File → Open → From finger
v. Click on the spectrum on the data slate
vi. Locate the peak position → pick the peak
vii. Mode tab → Diffusion Analysis → Apply
viii. Curve fitting and Diffusion coefficient will show up in the same window Fig.6