Diffusion Ordered Spectroscopy (DOSY) Overview

**BRUKER**

**DOSY** – Diffusion Ordered Spectroscopy. NMR diffusion experiments, like DOSY, are used to determine the diffusion coefficients of solute molecules of different sizes.

**1D DOSY** plots the signal intensity as a function of the delay. You can either vary the gradient and hold the delay (common) OR vary the delay and hold the gradient constant (rare).

**2D DOSY** plots the chemical shift on one axis and the diffusion coefficient on another. This allows you to separate multiple solute species.

**Table 1:**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Parameter Set</th>
<th>Pulse Program</th>
<th>Minimum NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D DOSY</td>
<td>1D-DOSY-dcif</td>
<td>ledgp2s1d</td>
<td>8</td>
</tr>
</tbody>
</table>

1. Lock, tune and shim.
   
a. Remember to tune the $^1$H channel (*wobb*)
b. DO NOT SPIN.

2. **Collect a good 1D $^1$H spectra.** DO NOT use *xaua* to start your acquisition. Type *rga*, let it finish, then *zg*.

**Experiment 1 (EXPNO)**

a. $^1$H: Acquire a 1D and reference the solvent peak. Zoom in and display all proton signals leaving 0.5 ppm of baseline on each side. Type *.setsw* (or click on the icon) to set the transmitter offset (o1p) and sweep width (sw).

b. Reacquire “reduced-sweep width spectra” with the number of scans (ns) needed to get good signal to noise and phase. Keep in mind that if you reduce the sweep width, you do increase acquisition time.

Write down the following values:

\[ \text{o1p: } \underline{\hspace{4cm}} \]
Type any parameter in the command line, hit **enter**, and TopSpin will display the value for you. You can change values here by highlighting the current value, typing in the value you want, and then hitting **enter**.

3. Load the 1D DOSY parameter set. You will have to run at least **TWO** DOSY 1D experiments, one at 5% gradient strength, the other at 95% gradient strength.

   **Experiment 2 (or any other new experiment)**

   ```
   rpar 1D-DOSY-dcif
   ```

4. Check pulse program (AcquPars tab) and make sure the correct one has been loaded (see Table 1).

5. Load the prosol parameters by typing **getprosol**.

6. Edit the basic parameters based on the information from the 1D $^1$H experiment (the values you recorded in **step 2**).

   - **ns**  Number of Scans
   - **o1p**  Transmitter frequency (ppm)
   - **sw**  Sweepwidth (ppm).
   - **sr**  Reference (Hz).

   Type **expt** and TopSpin will calculate the experiment time.
**5% Gradient Strength DOSY**

1. You will need to check the following parameters in the AcquPar tab.
   a. Gradient ratios:
      `gpz6` set this for a value of 5 for the 5% gradient strength 1D DOSY.

   Gradient Lengths:
   `p30` set this for 1000µs

   Recovery Delay:
   `d16` set this for 0.0001s

   Diffusion Delay:
   `d20` can be set for 0.1s. If your sample has low viscosity, you may want to set this for 50ms.

2. Set your `rg` value (determined in step 2 and recorded on page 2). Type `get prosol`. Start the acquisition with `zg`.

3. Process your first spectra with `ft` and `apk`. It should look much like a regular 1D ¹H spectrum. The goal in the 95% gradient strength 1D DOSY is to reduce the signal by 95%.

4. Once you have run the 5% gradient strength 1D DOSY, you need to run a 95% gradient strength 1D DOSY.

**95% Gradient Strength DOSY**

1. Increment the experiment number (`iexpno`).

2. You will need to check and set the following parameters:
   a. Gradient ratios:
      `gpz6` set this for 95 for the 95% gradient strength 1D DOSY.

      Gradient Lengths:
      `p30` set this for 1000µs

      Recovery Delay:
      `d16` set this for 0.0001s
Diffusion Delay:  
\textbf{d20} should be set to whatever you used in the 5\% gradient strength 1D DOSY.

2. Set your \textit{rg} value (determined in \textbf{step 2} and recorded on page 2). Start the acquisition with \textit{zg}.

3. Process your first spectra with \textit{ft} and \textit{apk}.
4. Use the multiple spectra overlay button:

This will display both the 5\% gradient strength 1D DOSY and the 95\% gradient strength 1D DOSY. Select whichever experiment you need by clicking in its name in the lower left window.

Adjust the signal intensities using these buttons:

You want the two spectra "match up" as best as possible. The scale should read 0.10 or less (but you should still see your peaks).

If the scale is not 0.10 or less, you will need to adjust (increase) the gradient length (\textit{p30}) and rerun the 1D DOSY until you have reduced the signal intensity (the scale is less than 0.10). \textbf{DO NOT EXCEED THE p30 MAX OF 2500}.

You are now ready to run a 2D DOSY experiment.
2D DOSY

1. Increment the experiment number (iexpno).

2. In the AcquPar tab change the Current pulse program (PULPROG) to \texttt{ledgp2s}. Click the following button:

   \begin{center}
   \includegraphics[width=0.2\textwidth]{image.png}
   \end{center}

   Change dimension from 1D to 2D.

3. The parameters should otherwise match your 95% gradient strength 1D DOSY.

4. Type \texttt{dosy} to create the gradient ramp function:

   
   \begin{itemize}
   \item Enter first gradient amplitude: 5
   \item Enter final gradient amplitude: 95
   \item Enter number of points: hit <enter>
   \item ramp type (l/q): \texttt{l} (linear)
   \end{itemize}

   \textit{Click OK to start the acquisition.}

5. Processing:

   The first thing you must do, on the spectrometer computer, is type \texttt{setdiffparm}. Save your data, and now you can process your DOSY offline in the computer room.

6. In the ProcPars tab, check the following parameters:

   SI is F1 should be a minimum of 256.
   \texttt{PH\_mod} (F1) should be no.
   \texttt{PH\_mod} (F2) should be \texttt{pk}

7. Type \texttt{rser 1} to phase the first serial file. Save as 2D and return.

8. Type \texttt{xf2}. You can do a baseline correction (\texttt{abs2}) but only if needed.

   You can go two ways from here: You can fit your curve and calculate your diffusion coefficient, or you can create a DOSY plot. You can also do both, but the best method for figuring out your diffusion coefficient is by fitting the curve.
Fitting the Curve

- Under the Analysis drop down menu, select *T1/T2 Relaxation*. This will open a flowchart on the right side of the screen.

- Follow the flowchart, starting with *Extract Slice*.
- *Extract Slice*: Select *Spectrum*. You can take any slice you want, but the first one is usually fine.
- *apk* and correct phasing as needed.
- *Define Ranges*: Follow the instructions on the screen. Only integrate the peaks that belong to your molecule of interest.

- Click the diskette button (Save region) and select *Export Regions to Relaxation Module*.

- In the *Relaxation Window*, you will see a curve. You can change the settings used to fit this curve by clicking the *Settings* button.
• You can select and delete points you do not want.
• It is recommended that you use the vargrad fitting function. Change other parameters as needed, click Apply, then OK.
• Click the Start Calculation icon. This should fit the function and provide you with your diffusion coefficient.

**DOSY Plot**

• eddosy. Change parameters in ProcPars as needed. Click the letter P to get to the regular processing commands. Click D to go to DOSY processing commands. You want to be in D.
• dosy2d to start the fitting and create your plot. Be patient, this can take some time.
• Click on the Spectrum tab to see your plot. Adjust the contour levels as needed.

• You can use the button and the crosshairs to figure out your diffusion constant (though this method is less accurate).
• Once you have created your DOSY plot, you can save, print, and otherwise treat it like any other TopSpin 2D spectrum.