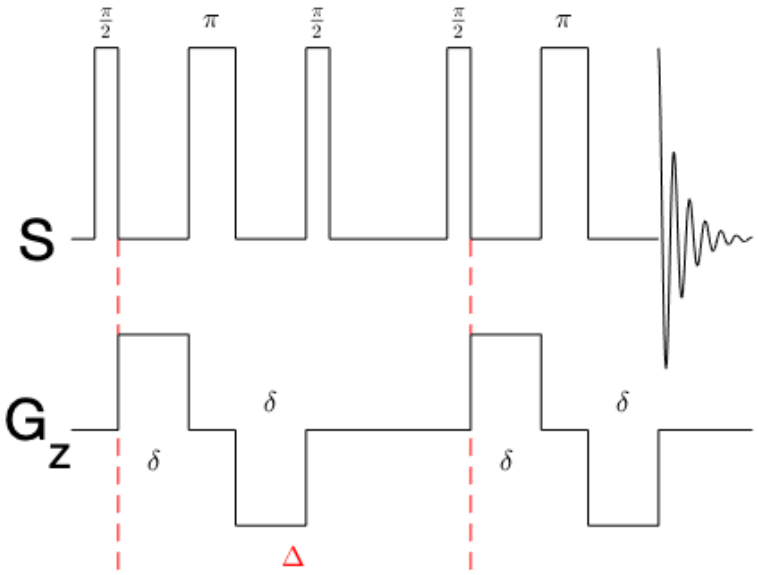
January 2023 NMR Topic of the Month: The DOSY Experiment



For what does the DOSY acronym stand?

DOSY = Diffusion Ordered SpectroscopY

What is the role of the DOSY sequence?

A DOSY experiment discriminates between signals based on molecular diffusion. The experiment is run many times with different gradient magnitudes, and a two-dimensional plot of diffusion coefficient versus chemical shift is produced.

How does the DOSY work?

Basic product-operators don't provide much insight, especially if the gradient's role is simply to remove all magnetization not along the z-axis. With the gradients removed, the sequence is just a spin echo that is then put back along the z-axis and then subjected to another spin echo.

So, clearly, the magic in this experiment lies with the gradient pulses. The gradients are all the same magnitude and duration (δ) but change sign on either side of the refocusing (π) pulses, so each $+G_z(\delta)\cdots\pi\cdots-G_z(\delta)$ may be thought of as a single $+G_z(2\delta)$ without chemical shift evolution. The first $+G_z(2\delta)$ encodes the initial z coordinates of the S spins, and the second $+G_z(2\delta)$ decodes the final z coordinates of the S spins. In between the two $+G_z(2\delta)$ the magnetization is kept along the z-axis, preserving it and allowing time for Brownian (translational) motions. Think of it this way: the first $+G_z(2\delta)$ labels the S spin as starting in a vertical region that has a size inversely proportional to the magnitude of the gradient, and the second $+G_z(2\delta)$ filters out the signal from any S spins not still in that same vertical region at the end of the sequence. In other words, if a spin is traveling quickly it will have changed its position significantly over the course of the experiment and its signal is lost with a smaller gradient magnitude.

What are the requirements for doing DOSY?

The DOSY experiment is one of the most gradient intensive experiments done in spectroscopy, and the gradient system should be well-calibrated or results will suffer.

Sample Preparation and Conditions

Once again, this is mission critical: you need a good sample. This means the synthesis/purification, sample handling, sample tube, *et cetera* all has to be good. During the experiment the sample needs to be temperature regulated, and kept in as consistent an environment as possible. If the temperature is significantly different from ambient, plan on using a

convection compensated version of the sequence. Never spin the sample for a DOSY experiment. Be sure to use a lock solvent and set the lock precisely, or you may see phase errors and poor fits as a result.

Delta Times

This experiment can take a while to set up and get correct. The first step is to look at the straight pulse-acquire spectrum. You need to be able to identify your peaks of interest in a few scans. This means they should be prominent and decently defined. Toward this end ^{1}H, ^{19}F, and ^{31}P are the only commonly chosen DOSY targets.

Next, DOSY experiments with a rough array of gradient magnitudes are done to look at the intensities and decay profile. The first goal is for the lowest gradient magnitude to produce 90-95% of the intensity of the straight pulse-acquire experiment. The second goal is for the highest gradient magnitude to produce 5-10% of the intensity of the straight pulse-acquire experiment. The two delta (δ and Δ) times are adjusted to meet these criteria *for the slowest moving peak(s) of interest*. The solvent peak, for example, will often drop-out much more quickly, but need not be taken into account (which also makes it a poor reference).

In terms of scale. δ is often 1 ms, and is followed by a gradient recovery time (not shown) that is always less than δ and usually is 0.5 ms. Δ varies greatly as the diffusion coefficient depends on molecular size and shape, solvent viscosity, sample temperature, *et cetera*. But always $\Delta < T_1$ and usually $\Delta > 50$ ms. The relaxation delay need not be quantitative (i.e. $5T_1$), but should be reasonable for the sample and do use steady state (dummy) scans. The gradient magnitudes should never be zero or at maximum output, but are often within 5% of these limits.

Finally, a finer (larger) array (often with more transients too) is acquired for the final DOSY. Ideally, all the signals (certainly those of interest) will decay away smoothly over the course of the experiment.

Processing and Interpretation

The t_2 dimension is processed using the usual Fourier transform, which may be done for each slice to produce a stack plot. The elements of the stack plot may then be fit to determine diffusion coefficients. Also the slices of the stack plot may be processed with a Laplace transform to produce a diffusion coefficient versus chemical shift two-dimensional plot. It's not quite as simple as that makes it sound, so consult the processing software manual.

> "Due to the difficulty in calibrating a perfectly linear gradient and conversion of DAC unit to Gauss/cm unit, and solvent viscosity and convection issues, when [an] accurate or absolute diffusion coefficient is desired, using an internal standard, ideally with a well known diffusion value, is very helpful so that all other diffusion coefficients can be scaled against it. However, for mixture separation, absolute diffusion value isn't important." – Hongjun Zhou, UCSB (https://nmr.chem.ucsb.edu/education/part7.html)

Dr. Zhou's comment is spot-on. It's not that you cannot get an absolute diffusion coefficient from a DOSY, it's just that it's really difficult to do. It is easier to get a relative diffusion coefficient, which is usually all that's important anyway. Buried in this comment is another truth: don't expect too much. Very often we are approached about using DOSY to discriminate between small differences between targets, and there's just no way a DOSY can answer questions such as these. The University of Manchester's NMR Methodology Group (https://nmr.chemistry.manchester.ac.uk/?q=node/29) has done many DOSYs, and many of their examples show well resolved targets when those targets are very different chemically. But even in their very capable hands, targets that are similar simply do not separate clearly (*e.g.*: the Phe-Val, Phe-Glu, Phe-Gly mixture in D_2O example from them available in the fidlib of VnmrJ).

References

1. D. Iverson, P. Bowyer, B. Heise, P. Sándor, *Agilent High-Resolution Diffusion-Ordered Spectroscopy (DOSY), User Guide*, G7446-90531, Agilent Technologies Inc., Santa Clara (2013).
2. R. Kesserbaum, G. Salnikov, *DOSY and Diffusion by NMR, A Tutorial for Topspin 2.0, Version 2.0.0*, Bruker Biospin GmbH, Rheinstetten (2006).
3. D.H. Wu, A.D. Chen, C.S. Johnson, *J. Magn Reson. Ser. A* **115**(2), 260-264 (1995).
4. C.S. Johnson Jr., *Prog. NMR Spectrosc.* **34**(3-4), 203-256 (1999).