Convection-Compensated DOSY on the Avance 360

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These notes describe the use of a prominent sequence used for DOSY work on Bruker spectrometers, dstebpgp3s. Because XwinNMR v2.5 is still the version being used on the AVANCE-360, the sequence has been modified, removing the use of gradient ramps (their proper use in XwinNMR requires v3.5 or newer; these newer versions need not only software but also hardware updates). The modified (simplified) sequence necessitates setting up a series of 1D experiments manually, and running them using **multizg**. These changes prevent processing the data in a standard fashion using Bruker's inverse Laplace transform algorithms (ilt). Changes allowing 2D acquisition could be made, with processing then possible, e.g., in MestreNova's Bayesian analysis.

The modified sequences follow Jerschow & Muller:[1, 2]

dstebpcc	[for unlocked samples, in protonated solvents], or
dstebpccl	[for locked samples, in ^2H solvents]

and incorporate all (or at least most) of the pragmatically useful "tricks" that have been found that enable the highest quality DOSY data to be obtained, including:

(1) ***ste*** – stimulated echoes: Magnetization is stored longitudinally, along the z-axis, after each 90°–180°–90° pulse sandwich. This storage allows longer **Δ** delays (**d20**) to be incorporated, limited only by T_1 relaxation. The other major variant, spin echoes, are limited by T_2 relaxation. The actual sequence uses a *double* stimulated echo (**dste**), canceling all constant-velocity effects (in combination with *cc* below).

(2) ***bp*** – bipolar gradient pulses: The gradients are switched positive to negative with a 180° spin echo pulse inserted in-between. The overall effect is that the two gradients dephase/z-axis encode in combination. Each gradient has a length **δ**/2 (**p30**), with the full gradient length then equaling **δ** (= **p30**×2).

(3) ***diff*** *(kappa* in VNMR) – unbalancing of the bipolar pairs reduces reliance on EXORCYCLE phase cycling during the sequence (as discussed by Pelta et. al.[3]). The positive gradient amplitude **gpz1** is set 10 to 20% larger than the negative amplitude **gpz3**.

(4) ***led*** – longitudinal eddy current delay: At the end of the sequence (2nd from last 90° pulse), magnetization is stored longitudinally, a crusher gradient applied (to remove any transverse magnetization), followed by a delay (**d21**) long enough to allow all eddy currents to become negligible. A final read 90° pulse is used just prior to acquisition, with confidence now that eddy currents will not distort the FID/spectrum.

(5) ***cc*** – convection compensation: This pulse sequence [A. Jerschow and N. Müller, J. Magn. Reson. *125* (1997) 372-375] minimizes magnetization decay due to translational motion arising from convection currents (laminar flow only) associated with temperature gradients across the sample. Convection is claimed to occur even close to ambient temps [*ibid*] due to heating from rf pulses. This compensation is a necessity for samples measured at temps away from ambient.

Steps for experiment setup:

1) Use a 5 mm probe, and make certain the gradient cable is correctly attached. [If convection continues to be a problem, use of a 3mm greatly reduces the actual convection; see notes to come later here or in the Varian DOSY write-up.] Leaving sample spinning on is asserted to reduce convection currents [J. Lunila et al, J. Magn. Reson. A *118* (1996) 50], although this has yet to be confirmed in our lab.

   Pulsatile heating as typically applied by NMR VT setups, is noted in some literature as generating convection, even for samples at ambient temps (see, for example, ref 3). It may be beneficial, therefore, to turn off temp control for experiments running at ambient temps.

2) Setup and acquire a standard 1D acquisition. Change parameters as required to obtain a high quality, quantitative spectrum. Of particular importance are:

   d1+aq $\rightarrow \geq 3\times T_1$ of the slowest relaxing nucleus of interest ($5\times T_1$ is better); **aq** need only be long enough to provide good (obtainable) resolution (and set **lb** $\geq 1/$**aq**)
   - *perform a T_1 estimate or quantitative experiment to confirm choices of **d1+aq***
   - perform a **paropt** on **p1** to measure the 360° (90°×4) pulse length

   ns $\rightarrow$ long enough to gain good sensitivity without making the final experiment too long; use **expt** to estimate the total time of the 1D experiment

   ds $\rightarrow$ ≥ 4; don't skimp here in the final set of experiments

3) Read in, **rpar**, the "DOSYHcc.UW" parameters set for 1H, or "DOSYFcc.UW" for F19 experiments. Use **eda** to change the pulse sequence to **dstebpccl** if working in a ^2H solvent and running under locked conditions. Change **d1, aq, sw, o1, ns, ds, p1, pl1** to match the 1D experiment taken in step 2 (can do a **wpar** to a new parameter set to allow simpler **rpar**s in future experiments).

4) Run a 1D experiment with the following primary parameter settings:

gpz1 = –gpz3 = 1	**;**typical range 1 to 80
d20 = 0.1s	**;**typical range 0.01s to T_1
p30 = 1ms	**;**typical range 0.5 to 5 ms
ns = 1 ds = 0	**;**use **ns** in multiples of 8, **ds**≥4

   [Other important parameters in the sequence involve:

p1 @ pl1	;critical this parameter be set correctly
p2 = p1×2	;180° pulse, requiring p1=90°
d16 = 0.5 to 1.0 ms	;gradient ringdown delay
d21 = 5ms	;LED delay
gpnam# = sine.100	;all gradients are sinebell shaped
gpz7 – 9	;crusher gradients, set as in pulse sequence
DELTA1, DELTA2	;computed to keep **δ/2=d21**, **Δ=d20** accurate

   Observe the reduction in signal from the 1D spectrum. Change to

   gpz1 = –gpz3 = 80

The desired result for this second experiment is signal intensities ca. 20% of the **gpz1=1** experiment. If the intensities are < 20%, decrease either **d20** or **p30** or **gpz1=–gzp3** as needed. If the intensities are > 20%, increase **d20** up to ca. the shortest T_1 of interest; after that, **p30** can be increased, but with limitations that are not well defined (**p30** ≤ 5ms for now). If the intensity is not decreasing much when **d20 ~ T_1**, **gpz1=–gpz3**=80 and **p30**=5ms, then diffusion in your system (solute+solvent at temp) is too slow to be accurately measured.

5) Once **d20** and **p30** are known, compute the gradient ramp, or array of gradient values, to use. [**gpz1** = 100 gives a gradient strength G_Z = **60 G/cm**, and the gradients are linear; i.e., **gpz1** = 1 → G_Z = 0.6 G/cm; calibration is needed to verify these numbers].

   Take an individual 1D spectrum for a set of seven or more values of **G_Z ≡ gpz1 = –gzp3**. Vary the values linearly in G_Z^2. If you've setup parameters having the largest **gpz1=–gpz3 = 80**, then use the following array/ramp:

   gpz1 = –gpz3 = 1, 20, 37, 49, 58, 66, 73, 80

   Unbalance the gradient pairs by adding ***diff*** = 0.1 to 0.2 to **gpz1**, with an identical reduction in **gzp3: → gpz1×(1+*diff*), gzp3×(1-*diff*).** If originally **gpz1 = –gpz3 = 70**, change to **gpz1 = 77.3 gzp3 = –62.7** giving the unbalanced pairs which reduce reliance on full phase cycling. Use the same ***diff*** factor (here 0.1) throughout the gradient ramp:

   gpz1 = 1.1, 22, 40.7, 53.9, 63.8, 72.6, 80.3, 88
   gpz3 = –0.9, –18, –33.3, –44.1, –52.2, –59.4, –65.1, –72

   When processing the data, use the original values as gradient amplitudes for each pair.

   – Another possibility is to vary **d20** across a set of experiments, and plot $\ln(I/I_o)$ vs **d20**. The two methods should give the same results, but variations in **d20** will involve T_1 and T_2 losses. The sequence tries to remove these, but by varying G_Z, relaxation losses are kept constant through the dataset. Thus, the preference is to vary **gpz1 ~ –gpz3**.

6) Remember to set **ns** = 8×i and **ds** ≥ 4; best to set these to obtain good signal-to-noise for a larger **gpz** experiment. Check the total time needed using **expt** , then multiplying by 8 (for gradient ramp as above).

   – ***Keep the gradient duty cycle ≤5%: failure to do so could damage the equipment.*** The G_Z duty cycle is the fraction of time the gradients are on during the experiment, e.g.,

   $G_{dutycycle}$ = (8×p30+3×p19) / (d1+d20+8×p30+3×p19+d21+aq) ≤ 0.05.

   Increase **d1** as needed to make the above true.

6) Setup each spectrum using **iexpno** , and change **gpz1 ~ –gpz3** (with unbalancing as shown above) in each exp manually. Acquire the dataset starting in the first expno and using **multizg**.

7) Determine the diffusion constant, *D*. Plot [from eq 6 in Jerschow]:

$\ln(I/I_o)$ versus **$-\gamma^2 \delta^2 G_Z^2 D [\Delta + (4\delta/3 + 3\tau/2)]$**

I = intensity (any resonance of desired compound)

I_o = intensity at very small gradient value (use $G_Z = 1$ data)

γ = gyromagnetic ratio = 4.258×10^3 s^{-1} G^{-1} (for ^1H; ratio freqs to get ^{19}F)

δ = length of the bipolar gradient pulse = **p30×2** (typically 1 to 10 ms)

G_Z = gradient strength ~ 0.60 G cm^{-1} × **gpz1**

Δ = time between pulses = **d20**

τ = gradient ringdown delay = **d16×2** (typically 1 to 2 ms)

The slope of the resulting line provides *D*. A typical result for an organometallic complex of MW = 600 is approximately 3.5×10^{-11} m^2 s^{-1}, for MW = 1200 is approximately 2.0×10^{-11} m^2 s^{-1}.

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1. Jerschow A, Muller N. (1997) Suppression of convection artifacts in stimulated-echo diffusion experiments. Double-stimulated-echo experiments. *Journal of Magnetic Resonance* 125(2): 372-5.
2. Jerschow A, Muller N. (1998) Convection compensation in gradient enhanced nuclear magnetic resonance spectroscopy. *Journal of Magnetic Resonance* 132(1): 13-8.
3. Pelta MD, Morris GA, Stchedroff MJ, Hammond SJ. (2002) A one-shot sequence for high-resolution diffusion-ordered spectroscopy. *Magnetic Resonance in Chemistry* 40: S147-S52.