DOSY/Diffusion on Avance III Spectrometers

last update: 22 August 2016 (cgf)

I. Introduction:

These notes complement Bruker's various documentation describing the setup of DOSY/diffusion measurements on Bruker AVANCE III spectrometers running TopSpin 3.x.* These experiments are nontrivial in experimental setup and in processing. cgfry also has a Bruker PowerPoint, available on request, that provides many useful pointers with respect to setup and processing.

A brief description of these topics is given below, followed by step-by-step instructions for setting up an experiment. Some discussion of processing follows. The data can be processed as a 2D dataset, with (typically) ^{1}H along F2 and D (diffusion constant) along F1: *this is the only form of display properly called* ***DOSY***. The same data can also be analyzed by fitting the decay in peak height or integral to obtain a decay time proportional to D: *this forms a* ***diffusion*** *analysis*. Both types of analysis and presentation should give the same results. DOSY and diffusion analyses start from the same, identical sets of raw data.

Many scientists prefer the presentation of DOSY, but diffusion curves often yield improved results due to a simpler and more intuitive method of judging the data quality. The "simpler and more intuitive" seems the other way around when looking at the graphs of the data — and bosses are often fooled by this presentation. One need only to ask how the different plots were arrived at, in terms of the mathematics used, to appreciate that the diffusion analysis is the much simpler analysis. Even so, the DOSY presentation is a powerful visual tool.

The complexity of DOSY analysis is readily demonstrated by comparing its implementation in TopSpin versus that in MestreNova. The Bayesian analysis performed by MNova, in cgfry's experience, is often terrible: it is great when it works, but is very poor in most other cases. *Thus, a strong recommendation is to use TopSpin for DOSY, and compare that to other methods of analysis (including MNova, but also diffusion analysis).*

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

dstebpgp3s(1d)	[with convection compensation (cc)], or
ledbpgp2s(1d)	[without cc]
ledbpgppr2d(1d)	[wo cc, including presat]
stebpgp1s19	[wo led, wo cc, includes 3-9-19 watergate]

A variety of other sequences are available, starting with dste*, ste* or led*. The 1st sequences two above incorporate most of the pragmatically useful "tricks" that have been found that enable the highest quality DOSY/diffusion data to be obtained. See section V for more detail.

* See in particular Bruker Help from within TopSpin: → Manuals → DOSY (in Applications section).

Convection compensation is a necessity for experiments measured at all temperatures away from ambient in 5mm tubes; it should always be used for experiments in cryoprobes with 5mm tubes. If convection is believed to be a problem in an experiment, even when using the convection compensated sequence: i.e., D is grossly wrong, or you see only a single diffusion constant rather than an expected two or three value:

a) Use of a 3mm tube greatly reduces the actual convection [see notes to come later here or in the Bruker DOSY presentation.]

b) Leaving sample spinning on is asserted to reduce convection currents [J. Lunila et al, J. Magn. Reson. A *118* (1996) 50], although experience here (cgf) suggests spinning causes as many problems as it cures (i.e., 3mm tubes are better, unless sensitivity is an absolute premium). Bruker states that Δ should equal a multiple of the spinning speed for best results.

c) Use of a Shigemi tube will decrease convection by reducing the temperature gradients across the now-smaller sample volume.

d) 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 (including the BCU chiller!) for experiments running at ambient temps.

II. Step-by-Step Experimental Setup:

A. 1D optimization of d20 (Δ) and p30 (δ):

1) Prepare the sample using guidelines as suggested above to reduce convection currents.

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 → ≥ $3\times T_1$ 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** ≥ 1/**aq**; lb=1Hz would be typical for ^{1}H diffusion experiments)

- *perform a T_1 estimate or quantitative experiment to confirm choices of **d1+aq***
- perform a **popt** on **p1** to measure the 360° (90°×4) pulse length if sample is has high salt

ns → long enough to gain good sensitivity without making the final experiment too long; use **expt** to estimate the total time of the 1D experiment; the 2d DOSY exps require **ns**=16×i

ds → ≥ 4; don't skimp here in the final set of experiments

⇨ Steps 3-7 optimize the dosy dataset to look like graph C above, by increasing or reducing the variables **d20** (Δ ≡ diffusion delay) and/or **p30** (δ ≡ diffusion gradient length):

3) **rpar** **H1_DOSYcc1d** to read in parameters for ^{1}H, or
H1_DOSYcc1d_presat to read in parameters for ^{1}H with presaturation, or
H1_DOSYFcc1d for ^{19}F experiments.

Note: Presaturation for peptides, proteins, etc., containing exchangeable protons is likely a good solvent suppression technique. The exchangeables will be attenuated, but they likely would not give the best Diffusion constants in any event: an amide proton spends some time on the peptide/protein, but then exchanges onto a water molecule. The measured D in this instance will be an average value between time spent as peptide/protein and water. So, reduce the water with presaturation, and use the protons on the molecule to determine D.

4) Change **d1, aq, sw, o1, ns, ds** to match the 1D experiment taken in step 2 (can do a **wpar** to a new parameter set to allow simpler **rpar** in future experiments).

Setup the 1D experiment with the following primary parameter settings:

gpz6 = 5	**;**typical range 2 to 95
d20 = 0.1s	**;**typical range 0.01s to *T_1[shortest]*
p30 = 1000µs	**;**typical range 0.5 to 3 ms *[stay ≤3ms!]*
ns = 2 ds = 0	**;**use **ns** in multiples of 2, **ds**≥4

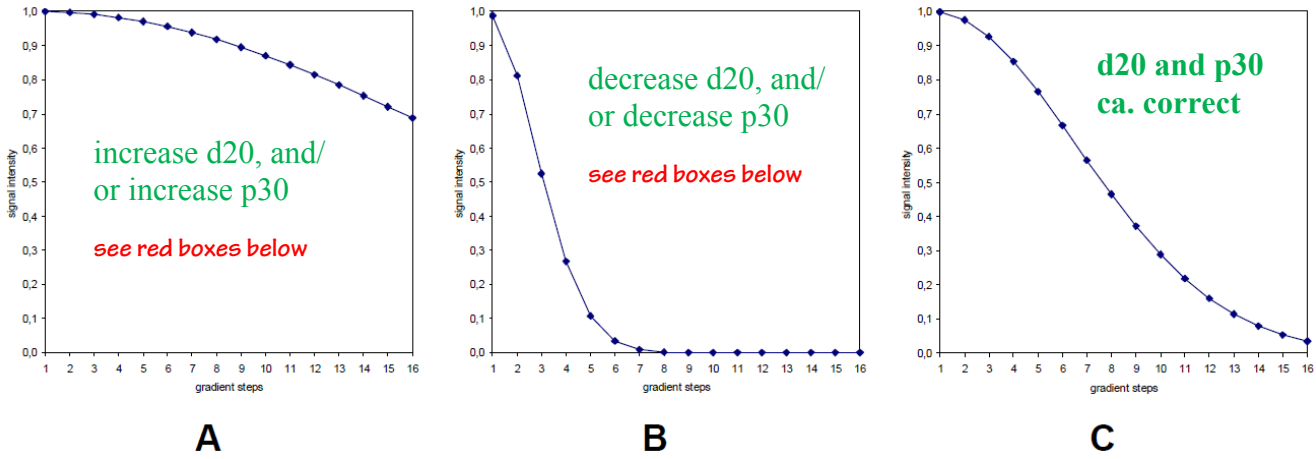
[Other important parameters in the sequence involve:

p1 @ pl1	;critical 90° be set correctly (OK if "normal" sample)
d16 = 0.2 to 1.0 ms	;gradient ringdown delay
d21 = 5ms	;LED delay
gpnam# = SMSQ10.100	;all gradients use this smoothed rectangular shape
gpz7 – 9	;crusher gradients, set as listed in pulse sequence
DELTA1, DELTA2	;computed to keep **δ/2=p30**, **Δ=d20** accurate

lb=1 absf1=1000,1000 absf2=-1000,-1000 absg=5]

p30 / (d1+aq) ≤ 0.05 is absolutely critical to prevent possible probe damage!! This equation makes sure the *gradient duty cycle* is ≤ 5%. NOTE: When **p30** = 2000, that is 2 msec (pulses are set in µsec). If d1=1 aq=1 (in secs), then the equation is computed as 0.002/(1+1) = .001; these parameters are fine.

6) Acquire a 1st spectrum with **gpz6 = 5**. Do **rga** first.



7) → Do an **iexpno**↵ .

Change **gpz6 = 95** and acquire a 2nd spectrum.

→ Use .md with the **gpz6 = 5** spectrum.

select the 2nd spectrum in .md and vertically increase and get scaling factor to ~20 (5%)

The desired result for this 2nd spectrum is signal intensities ca. 5% of the **gpz6=5** experiment.

a) If < 5% : decrease either **d20** or **p30** (these two 1st; can also decrease max **gpz6** value).

b) If > 10% : increase **d20** up to approx. T_1(shortest of interest); after that, **p30** can be increased, but keep **p30** ≤ 3ms (BBFO) or ≤ 2ms (cryoprobes).

c) If the intensity is not decreasing much when **d20** ~ $\boldsymbol{T_1}$, **gpz6**=95 and **p30**=3 or 2ms, then diffusion in your system (solute+solvent at temp) is too slow to be accurately measured: i.e., the compound's MW is too high, and/or the solvent viscosity is too high. *There is nothing you can do other than change solvent or temperature! Do not proceed otherwise!*

B. 2D setup and DOSY acquisition:

1) Once the optimized values for **d20** and **p30** are known:

a) **rpar** ***probe*_DOSYcc2d** to read in parameters for ^{1}H, or
***probe*_DOSYFcc2d** for ^{19}F experiments.

b) set **d20** and **p30** to the optimized values found in A.7

2) Set **ns = 16×i** i.e., an acceptable value as a multiple of 16. **expt**↵ will provide the total experiment time. Note especially the signal-to-noise of the **gpz6**=95 dataset you obtained, and keep **ns** large enough so reasonable quality data is obtained at this value.

Set **d1** = 2 to 5×$\boldsymbol{T_1}$.

3) ***Always*** run the au routine, even after an iexpno or wra:

dosy↵

This will setup the gradient array:

i) Use ≥ 7 (where 11 to 25 are more typical values).

ii) Run gradient amplitudes from 5 to 95%.

iii) The squared (q) setup is preferred.

III. Quick list of Processing Steps using TopSpin

A. Dosy workup:

edp↵ SI[F1] = TD[F1]*2	; TD[F1] = number of fids/gradients changes set in step 8
rser 1↵ efp↵ absn↵	; read 1st row from fid/ser, and process
.ph↵  **save↵** 	; phase, save to nD, return to 2D
xf2↵	; transform all fids in ser file (F2 only FT)
abs2↵	; polynomial baseline correct of order absg (dc correction by default; absg=1 straight line, etc)
setdiffparm↵	; moves Δ (=**d20**) and δ (=**p30**×2) into processing modules
eddosy↵	; opens dosy processing panel
dosy2d setup↵	; does run-through of data, and estimates D range
dosy2d↵	; performs the dosy transform as setup in eddosy panel
new↵	; increment PROCNO by 1 to retain different processing sets (which can then be **.md** compared)

in eddosy changing **PC** to larger number may help (e.g., **PC**=10 or 40)

B. Diffusion workup:

use **Analyse → T1/T2** (see Bruker DOSY manual; pg 19)
something like

xf2↵ abs2↵ [might change absg=1 or 5 and see effect]
setdiffparm↵
Analyze → T1/T2 → FID → Spectrum → 1
back to **T1/T2 → Peaks/FID → Integration**→ integrate regions of interest →
save to Relaxation module
Relaxation → 
click on **sq lg** in that order in Relaxation to get linear plot

IV. Comments about DOSY/Diffusion processing in MNova:

All the processing above can be done similarly in MNova. Ease-of-use is clearly better in MNova, but that should not sway the user away from TopSpin for DOSY analysis.

- MNova uses a Bayesian algorithm for the DOSY (2D) transform. There are advantages and disadvantages to this technique. Good values are found (in our experience) for major components. But the Bayesian technique produces considerable noise along all rows having D values having higher probabilities. The technique then completely fails when one is interested in minor components, and can lead to absurd results. **We often find Bruker's DOSY transform to be clearly superior.**

- Bruker's DOSY methods have many options and algorithms (see **eddosy** for a listing, and Bruker's manuals). There is very little one can change in MNova. The recommendation is to always use a few techniques that appear suitable, both Bruker and MNova, to obtain the best idea of the quality of the data. This includes using "diffusion" of T1/T2 analysis of the data (which cgf strongly recommends).

- Both Bruker and MNova do reasonable jobs with "diffusion" plots. But Bruker's analysis via T1/T2 does a number of things that are odd and hard to justify: it removes points it considers too noisy or poor (and difficult to understand when and why); it does, we believe, an error analysis during the fit and therefore consistently fits above the last set of points (is this good or bad?).

- MNova's error analysis is simpler to work with, and provide nice export (copy-paste) features to get the data into Excel.

Other notes:

The normal method of acquiring DOSY data is to vary the gradient amplitude, accomplished by the **dosy** au routine.

- Another possibility is to vary **d20** (Δ) 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 **gpz6**.

1) Remember to set **ns** = 16×i and **ds** ≥ 4; best to set these to obtain good signal-to-noise for the **gpz6=95%** experiment..

   - ***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.,

     $$\mathbf{G_{dutycycle} = (8\times p30 + 3\times p19) / (d1 + d20 + 8\times p30 + 3\times p19 + d21 + aq) \leq 0.05}.$$

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

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

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

   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**

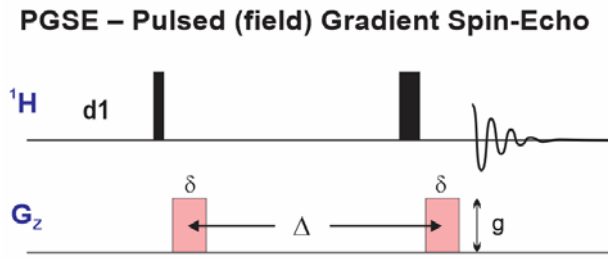
   Δ = time between pulses = **d20**

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

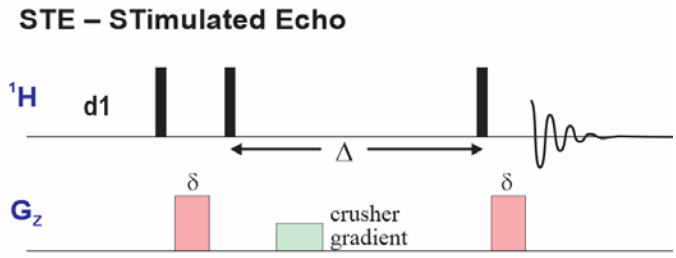
   The slope of the resulting line provides *D*. A typical result for an organometallic complex of MW = 600 is approximately 3.5×10^{-11} m^2 s^{-1}, for MW = 1200 is approximately 2.0×10^{-11} m^2 s^{-1}.

V. Pulse sequence details

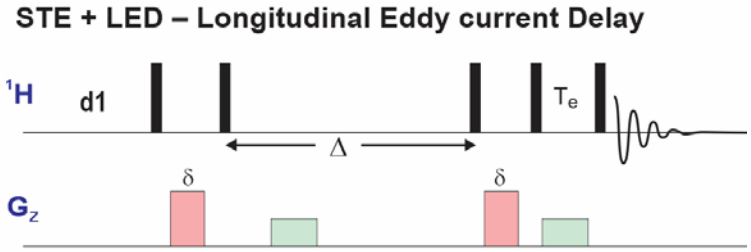
The first sequences used for diffusion measurements involve simple spin-echo or gradient-echo components. Needing quantitative intensities, preparation using long enough **d1** is important. The length, δ (p30), and amplitude, **g** (gzp6), of the gradient is critical, as is the primary diffusion delay, Δ (d20).



A few serious problems and limitations quickly became apparent. One limitation is $\Delta < T_2$ to prevent too significant loss in sensitivity. The gradient pulses cannot be asymmetric with the 180° pulse if chemical if an issue. And J-coupling will impact the experiment. A number of improvements have been added, as follows:

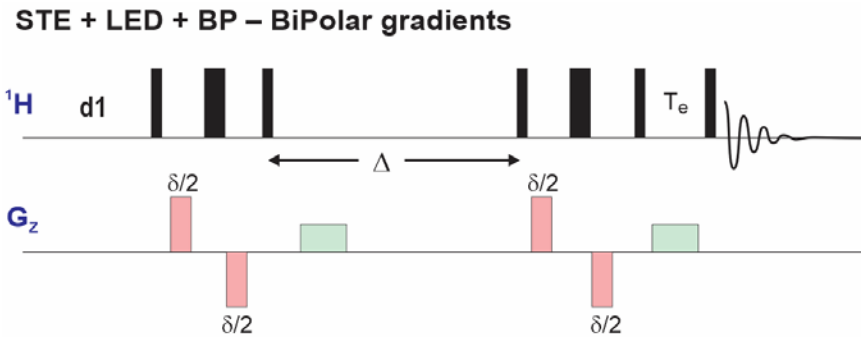


(1) ***ste*** – The issues with PGSE are improved by splitting the 180° pulse into two 90° pulses; the sequence still echoes magnetization via STimulated Echoes. Magnetization is stored longitudinally after the 2nd 90° pulse. This storage allows longer **Δ** delays (**d20**) to be incorporated, limited by T_1 relaxation, rather than T_2, as in the PGSE sequence. Crushing transverse magnetization during Δ eliminates a lot of other artifacts, and reduces chem shift and J dependences. The convection compensated sequence uses a *double* stimulated echo (***dste***), canceling all constant-velocity effects (see *cc* below).



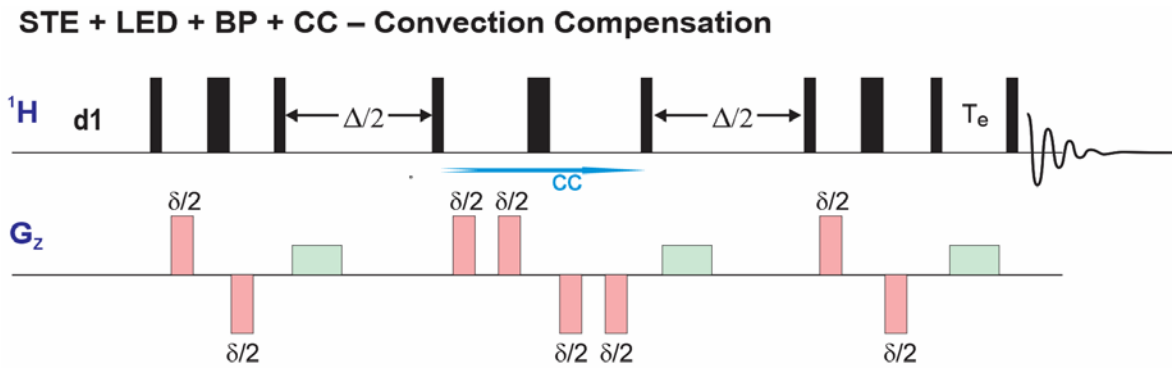
(2) ***led*** – longitudinal eddy current delay: At the end of the STE sequence, magnetization is once again stored longitudinally (along the z-axis). A crusher gradient applied (to remove any transverse

magnetization), followed by a delay T_e (**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.



(3) ***bp*** – bipolar gradients: Eddy currents are further reduced by using pairs of gradients that are opposite in sign. A 180° pulse in the middle enables the gradient pair to act in concert: they both dephase, or both re-phase, the magnetization. Each gradient pulse has a length δ/2 (**p30**), with the full gradient length then equaling δ (= **p30**×2).

(4) ***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]). At this time, we're (cgf) not sure how useful this implementation is in TopSpin 3.x, and have not pursued in on Bruker spectrometers.



(5) ***cc*** – convection compensation: This pulse sequence[1,2] minimizes magnetization decay due to translational motion arising from convection currents (laminar flow only) associated with temperature gradients across the sample. *Convection currents will completely ruin DOSY/diffusion experiments if not properly dealt with*, and occur even close to ambient temps due to heating from the VT controller and from rf pulses (e.g., decoupling in a $^{19}F/^{31}P/^{13}C$ experiment, or from the spinlock in a TOCSY experiment). **Convection compensation is a necessity for experiments measured at all temperatures away from ambient in 5mm tubes.** 50% of the magnetization is lost in this experiment compared to the non-*cc* versions. With cryoprobe use, samples are have convection occurring at ambient temps; thus *cc*-sequences are prudent to run for most experiments.

† Just as an electrical current in a loop creates a magnetic field, changes in magnetic fields will create currents in circular paths of wire and other metal structures. The currents produced during a pulse-field gradient are called eddy currents. Eddy currents are pernicious in diffusion and imaging studies, as they can persists for many milliseconds, create a magnetic field feedback, and thus distort the detected signal.

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.