Diffusion Measurement By NMR

Background

Diffusion measurements in NMR refer to translational diffusion, not rotational diffusion nor to "spin diffusion". For random, Brownian motion, of a spherical molecule, the Stokes-Einstein relation is:

$$D = \frac{kT}{6\pi\eta r_s}$$

where η is viscosity, T is temperature, r is the hydrodynamic radius, and D is the diffusivity.

Diffusion is measured directly with an echo-based pulse sequence. The first part of the diffusion pulse sequence spatially labels, with a phase factor induced by gradient pulses, spins along the NMR tube's z axis. There is then a rather long delay, Δ, typically 50-200 ms, during which diffusion occurs. Finally, another gradient pulse refocuses (or "undoes" the spatial labeling) the signal. The greater the displacement of the spins during Δ due to diffusion, the less refocused the signal and the more reduced the signal intensity. The following equation describes the situation for *random isotropic diffusion*. (Deviations are to be expected for other cases!) I is the intensity of a peak, D is the diffusivity, g is the pulse gradient strength, δ is the length of the gradient pulse, and Δ is the diffusion time.

$$I = I_0 \, e^{-D\gamma^2 g^2 \delta^2 (\Delta-\delta/3)}$$

In a typical experiment, the software creates a series of values of g, ranging from 95% to 2% of the maximum gradient strength (53.5 G/cm on our spectrometer). Plotting I vs g for a given peak then gives a diffusion curve which is fit by software to give D. The following plots, taken from Bruker's DOSY and Diffusion handout, describe three possible diffusion curves. Plot C is ideal while plot A and B do not sample the curve well and thus would not give reliable fits. For cases A and B, you must adjust Δ and/or δ and repeat the experiment. For the situation described by plot A, insufficient intensity reduction has taken place. To correct this, either Δ and/or δ must be increased. For plot B, too much intensity has been lost and either Δ and/or δ must be decreased.

A   **B**   **C**
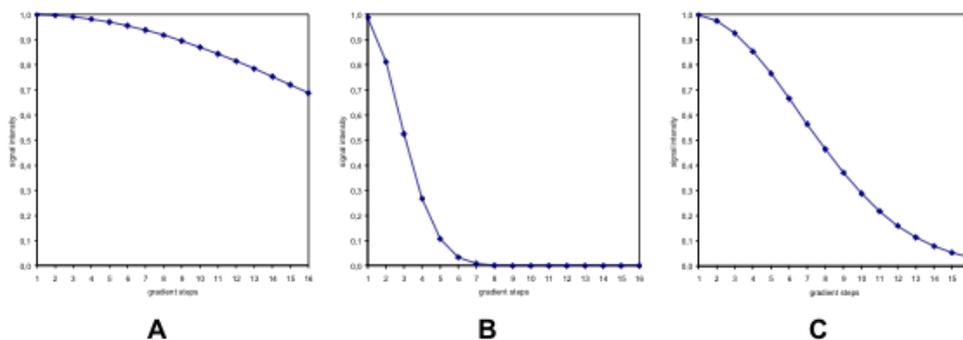
The maximum value of Δ depends on the T_1 (for "stimulated echo" sequences, which is standard) for your protons. If Δ exceeds T_1, then there is little signal left at the end of the sequence. There exists a lower limit on what diffusivity is measurable with our equipement. As molecules become bigger or are in more vicous fluid, their diffusivity decreases, and ideally, the value of Δ is increased to compensate. However, the T_1 of larger molecules is shorter as well and thus it may not be possible to increase Δ.

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It is useful to distinguish DOSY and diffusion. While diffusion is measured in both cases, DOSY refers to a 2D plot where the X axis is chemical shift, and the second (Y) axis is diffusivity. Diffusion analysis refers to fitting the decay in peak height or integral to obtain a decay time proportional to D for one peak and one diffusivity at a time. Plots like those above are obtained. Both types of analysis and presentation should give the same results. DOSY may be an easy to interpret presentation but it can mask problems caused the complexity of the mathematical analysis needed to generate the second axis. When there is more than one component with different diffusivities *and the components have the same chemical shift*, DOSY can attempt to deconvolute the individual contributions. When there is only one diffusing component at each chemical shift, however, I recommend a simple 1D diffusion analysis, instead of 2D DOSY, since it offers the best precision and accuracy of measuring diffusivities. *Simple 1D diffusion analysis using Topspin's T1/T2 routine is described below in this handout.* Both Topspin, MestreNova, and Bruker's Dynamic Center can perform 2D DOSY analysis. Bear in mind that DOSY is a processing only procedure. That is, data collection proceeds exactly the same for both so both analyses can be done, if desired. Please John Decatur for assistance with 2D DOSY analysis.

A potential problem is convection. When there are temperature variations over the sample, convection will lead to incorrect, over-estimated diffusivities. To limit convection issues, make sure your sample is only 600 µL so that your sample is within the dewared region of the probe. In addition, make sure the temperature controller is actively controlling the temperature. Using Shigemi or 3 mm standard tubes is helpful. The presence of convection can be confirmed if the diffusivity varies with Δ, the diffusion time. Convection compensated sequences exist, but they are less sensitive. When doing diffusion at temperatures that are not room temperature, the convection-compensated versions must be used. The most commonly used Bruker pulse programs are:

> stebpgp1s – not convection compensated (default)
> dstebpgp3s – convection compensated

Almost always, diffusion experiments use ^1H detection. ^{19}F is also possible but other nuclei, such as ^{13}C, face serious difficulties. The following assumes ^1H detection.

Another problem that plagues diffusion data is that the phase often changes from spectrum to spectrum, which leads to scatter in the intensity data. My sequences are modified from Bruker's standard sequences and largely remove these phase instabilities – the reason is the gradient pulses are "lock compensated".

Setup and acquisition

The Bruker Topspin software treats the diffusion experiment as a pseudo-2D experiment. When doing a diffusion experiment, the 2D window will be present. It is actually an arrayed 1D experiment but that is very similar to a 2D experiment. One can look at the individual 1D spectra using the 2D commands for reading in "slices".

- Do not spin the sample. Lock, shim and take a normal 1D spectrum. Be sure to tune the probe (atma command). Determine the spectral region to be used. Note the value of rg found by rga or start.
- Type new to change the filename, or preferably, the experiment number.
- Type rpar diffusion.top to read in parameters for the diffusion experiment.
- Adjust the following parameters that are specific to the diffusion experiment
    - Δ = D20   the time over which diffusion occurs. Set to 0.05-.2 sec.
    - δ =P30*2 length of gradient pulse. Set to 1 – 5 msec. Thus P30 is half the total δ.
- Enter the value of rg determined in the 1D spectrum above.

Other parameters

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You must also set the following:
ns – should be a multiple of 4
d1 - relaxation delay -should be 4 or 5 times T1. 10 seconds is a typical value.
sw, o1p, - these and any other standard parameters should be set for your particular spectrum

1. Type dosy to create the needed gradient values. You will be asked a series of questions. Initial gradient value should be 5, final value 95. The number of points can range from 8 to 20 or so. For ramp type, choose q, for quatratic. You may select start directly from this macro. Or,

2. Type expt to see how much time the experiment with take. Type zg to begin acquisition.

Processing

3. Once acquaition is finished, type setdiffparm, which transfers the diffusion parameters above into the processing software. If you neglect this step, the t1/t2 routine does not know how many points exist nor what gradient strengths used to create them.

4. Phasing, peak picking and integration. The phasing must first be determined and that is easiest on one of the spectra that is only partially attenuated. Type rser 1 or rser 2. Type ef, and phase the spectrum but when you save the phasing, click on save as nD [image] and then [image]. Do not close the 1D spectrum. You must now define the peaks that you want picked for their diffusivities. Click on Peak Picks and select manual picking mode, [image]. Pick as many as you wish and click [image] and select "export regions and biggest peak within region to relaxation module and .ret.". Then [image]. Then, click on integrate, define integrals around the peaks you picked above, and then under [image] select " Export Regions To Relaxation Module and .ret"

Close this spectrum by clicking on its x box in its upper right corner. You should be back at the 2D interface.

5. Type xf2 to transform all the spectra.

NOTE: at this point, you may choose to analyze your data with Bruker's Dynamic Center software. To do so, follow the directions in the Kinetics handout instead of what is below.

6. It is very useful to inspect your data prior to further analysis. Under Process, Advanced, select Extract Rows/Columns. Click row and using the middle mouse button, select a row for inspection. Check the box next to "Interactive row/column display"and then OK. Select [image] to display rows. As you move the mouse across the 2D spectrum, different rows are displayed.   In this way, you can see if peaks are attenuated due to diffusion and whether or not the spectrum phase is constant. When finished, click return, [image].

7. The next step is to have the software choose the intensities from each of your spectra. Type t1/t2. Your points should be automatically picked. It is almost always necessary to reset the view display region by clicking [image]. To pick them manually, click [image]. To adjust fitting parameters, click [image]. In particular, make sure the function type is set to vargrad. If your points are not all being picked, you may also need to increase the number of drift points to 20 or so and then re-pick. Click [image] to calculate the diffusivity. If the fitting fails, usually it is because the initial diffusivity value, is far from actual. (or you may not have unrestricted diffusion, which is a more fundamental problem) To change the initial value, click Guesses, [image], and change Guess D. To delete a particular, possibly outlying point, right-click over the point and select delete. If you have picked more than one peak in your spectrum, click + or – to change to the next peak. Click [image] to generate a report.

Notes about picking peaks. You have two choices to pick your peak intensities:

a) pd searches over a specified range (drift points) to find the maximum intensity. This is necessary if the chemical shift maximum drifts slightly over the course of the experiment. Sometimes, however, the pd command fails to pick all peaks and the drift points can be increased.

b) Pd0 picks the intensity at the exact frequency you defined in the peak picks routine.