Proton DOSY Experiment

Introduction

The **DOSY** (**D**iffusion-**O**rdered **S**pectroscop**y**) **experiment** provides accurate, noninvasive, molecular diffusion measurements on biofluids, complex chemical mixtures and multi component solutions. In DOSY spectra, chemical shift is along the detected F2 axis and diffusion coefficient is along the other F1 axis.

Molecules in the solution state move. This translational motion is known as Brownian molecular motion and is often simply called diffusion or self-diffusion. Molecular diffusion depends on a lot of physical parameters like size and shape of the molecule, temperature and viscosity.

Pulsed field gradient NMR spectroscopy can be used to measure translational diffusion. By use of a gradient pulse, molecules can be spatially labeled. After this encoding gradient pulse (δ), molecules move during the diffusion time (Δ). Their new position can be decoded by a second gradient pulse. This encoding/decoding procedure results in an attenuation of the NMR signal which can be described by the following equation:

$$I(g) = I(o)\exp\left[-(\gamma g\delta)^2 D\left(\Delta - \frac{\delta}{3}\right)\right]$$

Where **I** is the observed intensity, **D** is the diffusion coefficient, **γ** is the gyro magnetic ratio of the encoded nucleus, **g** is the gradient strength, **δ** is the length of the gradient pulse, and **Δ** as mentioned previously is the diffusion time.

The diffusion experiment records a series of 1D ^{1}H spectra at increasing gradient strengths (g) and then fits the signal intensity decay to the above equation to obtain **D**.

Convection within the sample tube, such as, moving liquid columns along the sample axis (primarily due to temperature gradients), can seriously affect diffusion experiments, in particular, at elevated temperatures. Convection currents are caused by small temperature gradients in the sample and result in additional signal decay that can be mistaken for faster diffusion.

DOSY uses three parameters to define the duration of the diffusion: gradient length **δ** (**P30** in topspin, the total gradient defocusing time), the diffusion gradient level **g** (**GPZ6** in topspin, maximum 95%), and the diffusion delay **Δ** (**D20** in topspin, 60 ms as default, max determined by the shortest T1 relaxation). In most case, **GPZ6** is the variable parameter to be arrayed for DOSY purpose. Depending on sample, you might need increase **D20** and/or **P30** (max **2ms**!) in order to obtain enough signal attenuation. The purpose of doing this is to get a diffusion decay curve like in the **figure C** below that will give you the best DOSY fitting.

There are two sets of parameter files under user directory:
ledbpgp2s (“longitudinal eddy current delay" LED-bipolar gradients pulse sequence)
dstebpgp3s (double stimulated echo for ***convection compensation*** and LED using bipolar gradient pulses for diffusion using 3 spoil gradients).

With LED, magnetization is stored along the z-axis during most of the pulse sequence, so T1 relaxation is predominant. Since in macromolecules the T1 relaxation is slower than the T2 relaxation, the LED experiment is better suited to the measurement of Ds of slower diffusing molecules where longer "diffusion delay" is required to detect attenuation of the signal.

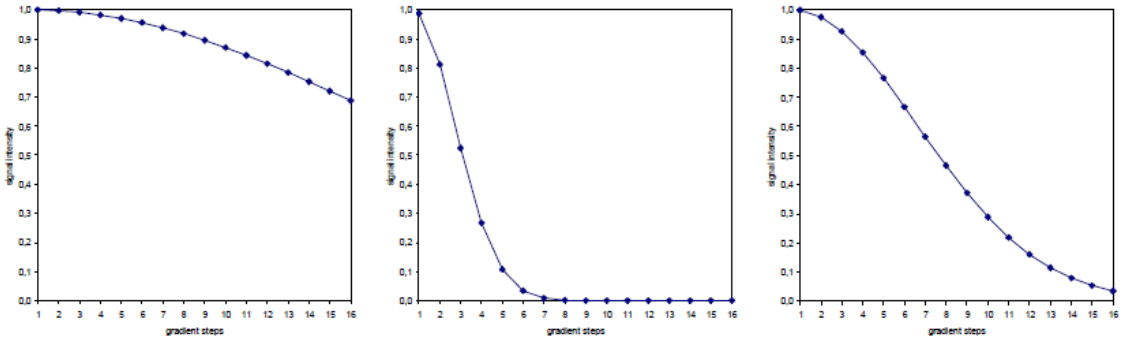

Figure: Simulated diffusion decay curves by varying the gradient strength **g** from 2 to 95% in 16 steps for the same diffusion constant, but with different selection for Δ and δ. They are chosen too small (A), too big (B), and properly (C) to sample data points along the whole decay curve.

Experiment

The DOSY pulse program used in the following procedure is the Stimulated spin-echo experiment using bipolar gradients and an additional delay just prior to detection for the ring-down of any possible eddy currents (**ledbpgp2s**). The same procedure works for **dstebpgp3s** if you need convection compensation.

1) To set up a DOSY experiment, start with recording a normal proton spectrum, followed by optimizing **P1**, **SWH**, and **O1**, if necessary.
2) Type "**rpar ledbpgp2s1d_nu all**" to retrieve 1D dosy parameters (or "**rpar**" to select "**ledbpgp2s1d_nu**"). Update solvent with yours (default is CdCl3)
3) Check to make sure the **P1**, **SWH**, and **O1** are same as your proton experiment. The recycle delay **D1** should be 1-2 T1 and dummy scan **DS** should be at least 8. Adjust **NS** accordingly to give sufficient S/N.
4) Change **GPZ6** to 2% and type "**zg**" to collect data.
5) Use "**edc**" to create another 1D experiment and change **GPZ6** to 75% and type "**zg**" to collect data
6) Click  (dual display) to compare the 1D data with **GPZ6** at 75% to the previous 1D of 2% to check if the nmr signals of interest are attenuated to less than 5-10% of the intensities obtained with **GPZ6** at 2%. If you don't get there or already past it, adjust **GPZ6** (to 95% or 50%) accordingly to make sure you get there. Write down the **GPZ6** value for 2D DOSY setup.
7) If changing **GPZ6** alone is not enough to attenuate the signal enough, increase the **D20** and/or **P30** to achieve the goal.
8) Type "**rpar ledbpgp2s_nu all**" to retrieve 2D dosy parameters (or "**rpar**" to select "**ledbpgp2s_nu**"). Update solvent, **P1**, **SWH**, and **O1** with the values from your proton experiment
9) Type" **dosy"** to create the gradient ramp function:
   Enter first gradient amplitude: 2
   Enter final gradient amplitude: 95 (or the value obtained from 1D DOSY)
   Enter number of points: 16 (or the number you think appropriate for your sample)

ram type (l/q): l

and finally, **Do you want to start acquisition?** Select **OK** to collect 2D DOSY data.

Processing

1) Set the proper window function.
2) Type "**eddosy**"
3) Type "**setdiffparm**" (or click )
4) Type "**xf2**" (or click )
5) If you need phase the spectrum, type "**rser 1**" to read the 1st fid to a new prono and type "**efp**" and "**apk**" to get correct **PHC0** and **PHC1** numbers. Then go back to 2D DOSY dataset and correct the phase values. Remember the phase mode is "**pk**" for direct dimension (F2).
6) Type "**dosy2d setup**" (or click )
7) Type "**dosy2d**" (or click ), you should see the 2D DOSY spectrum with chemical shift along the detected F2 axis and diffusion coefficient along F1 axis.

Additional notes

Sample preparation: make sure the sample volume is not more than 550 ul.