Quantitation in NMR, T1, Relaxation Delay, and Repetition Time:

<u>Question</u>: What do I need to use for a relaxation delay to obtain quantitative integrations?

<u>Answer</u>: *It depends upon how "quantitative" you wish to get, what your excitation pulse-angle is, and on the longest T1 relaxation time in your sample.*

If we define "Q" as the "quality" desired for quantitation, often called "M_z/M_0" (1.0 would mean that the steady state magnetization = the equilibrium magnetization...in reality, Q will always be < 1.0), the relationship can be given by:

$$Q = \frac{1 - e^{-Tr/T1}}{1 - \left[e^{-Tr/T1} \cdot Cos(\alpha)\right]}$$

- **Tr** is the repetition time (Acquisition time + Relaxation Delay). On the Varian instrument, **Tr = at + d1** (on a Bruker Tr = **aq** + d1)
- α is the excitation pulse angle. On the Varian instrument, the command "pw(α)" will set the angle to α. On a Bruker, it depends on the pulse sequence used.

Two more convient forms of the above equation are shown below:

$$Tr = -\ln\left(\frac{1-Q}{1 - Q \cdot Cos(\alpha)}\right) \cdot T1 \quad \text{...or solve for } \alpha \text{ ...} \quad \alpha = \cos^{-1}\left(\frac{Q - 1 + e^{-Tr/T1}}{Q \cdot e^{-Tr/T1}}\right)$$

After choosing on the "quality" of integrations (based on relative relaxation rates), use the known (or estimated) longest T1 in your sample. Using the left equation above, enter the pulse angle (the default in the standard ^{1}H parameters on our instruments is 30 degrees), and calculate the minimum value of Tr necessary. Or you can use the right equation and enter Tr, the longest T1, and calculate the pulse angle (α) that will give the desired "Q".

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Example: We want our integrated intensities to be accurate to within 95% (Q=.95), and we estimate that our T1s would be typical for a non-degassed organic sample (T1=1-3 sec.). Our excitation pulse is 30^0. Using the above equation: Q=.95, T1=3.0, $\alpha=30^0$...

$$Tr = -\ln\left(\frac{1 - .95}{1 - (.95 \cdot Cos(30))}\right) \cdot 3.0$$

This gives Tr = 3.8 Sec.

The default acquisition time is 3.0 sec (at=3.0), so the relaxation delay necessary for 95% relaxation would be <u>0.8 seconds</u>.

The *Ernst Equation* (Ernst *Angle*): If you don't care about quantitation, but rather want to achieve the best overall Signal:Noise ratio for a slowly relaxing nucleus (such as an unprotonated carbon), you can calculate the optimum flip-angle (α) if you know Tr (d1+at) and you can estimate the T1 of the nucleus.

$$\cos(\alpha) = e^{-Tr/T1}$$ At the end of the experiment, the relative integrated peak intensities will not be quantitative, the ultimate S:N of the slowly relaxing carbons will be improved, and the overall S:N of the quickly-relaxing carbons (T1 is small) will be lower because there is less signal-per-scan for these rapidly-relaxing nuclei . Note that when α is very small, the signal-per-scan becomes vanishing small; therefore; one can increase the d1 delay to keep the *Ernst angle* > 20-degrees so at least 33% of M0 is detected.