

# How to calibrate the $^1\text{H}$ $90^\circ$ Pulse on the Varian NMRs

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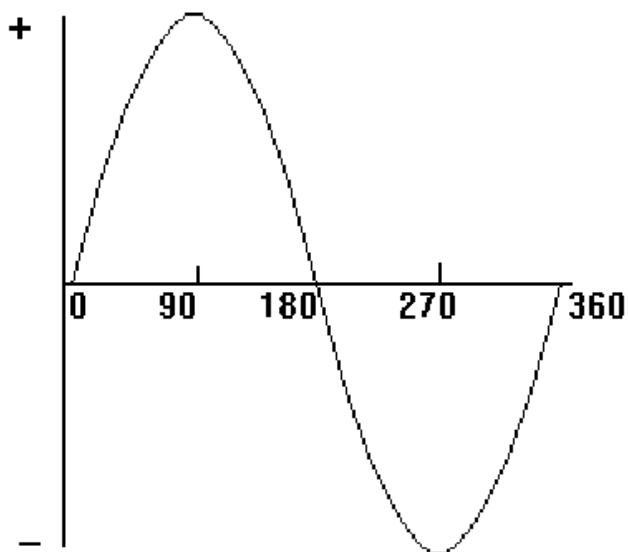
## Background Information:

For many NMR experiments such as DEPT, TOSCY, NOESY, and HMBC, the pulse sequence requires that many specific pulses or a series of pulses ( $90^\circ$ ,  $45^\circ$ ,  $180^\circ$ , etc.) be applied. Without properly calibrated pulses, many of these experiments will yield meaningless results, or most likely, fail outright.

Since each compound (and each nucleus) has a different chemical environment, each had a distinct  $90^\circ$  pulse width (pw90). The pw90 is defined as the duration, in microseconds, that the rf signal must irradiate your sample in order to tilt the magnetizations into the XY-plane,  $90^\circ$  away from the Z-axis of the NMR's magnetic field. Another way to think of it is how long you must pulse in order to tip all the spins into the XY plane. This pulse is often referred to as the  $\pi/2$  pulse.

The  $90^\circ$  pulse width for proton NMR experiments is about 10-20 microseconds on most modern spectrometers. The exact value of the  $90^\circ$  pulse width depends on the sample (nucleus, solvent, etc.) as well as the instrument (probe, transmitter power, etc.). It may be 5 microseconds long, 17 microseconds, or 35 microseconds, or some other number determined experimentally. For this reason, it is necessary to measure the  $90^\circ$  pulse for every sample you need to perform experiments on. Lucky for us, the proton  $90^\circ$  pulse is typically quite similar for all the protons in your sample.

Measuring the  $90^\circ$  pulse width is simple enough. Remember that the  $90^\circ$  pulse tilts the sample magnetization into the XY plane, which contains the detector. A simple pulse sequence of irradiate-observe (in Varian lingo this is called *s2pu*) should show a maximum for the pulse duration corresponding to a  $90^\circ$  pulse. Because it is difficult to discern maximum signal intensities by comparing similarly intense peaks (i.e. comparing an  $89^\circ$ , a  $90^\circ$ , and a  $91^\circ$  pulse.), we look at the  $180^\circ$  or the  $360^\circ$  pulse.



The  $360^\circ$  pulse corresponds to a 'null' - no signal is observed at this irradiation. Searching for this null is easier to determine and has the added advantage of minimizing the time required between pulses due to relaxation issues.

## The nitty gritty:

Please note that **all bold print** are input into the command window. Many steps are being skipped on the assumption that you already know how to acquire a simple 1D-proton spectrum.

- Tune the probe and obtain a well-shimmed  $^1\text{H}$  spectrum.
- Use the cursors to zoom in on an area of interest and carefully phase this portion of the spectrum. If necessary, phase this portion by hand.
- Prepare the instrument by typing **nt=1 gain='y' ai vp=50** (number transients = 1, receiver gain set to value in stored experiment, set absolute intensity mode, spectrum vertical position = 50)
- Type **pw90?** and remember this value (write it down!). For this example, let's assume this value to be  $8.65\mu\text{s}$  (microseconds).
- Create an array of values bracketing the  $360^\circ$  pulse width ( $4 * \text{pw90}$ ). For example:
  - Let's assume  $4 * \text{pw90}$  is  $34.6\mu\text{s}$ , but we of course would like to more precisely determine this value.
  - Type **array('pw',20,32.6,.2)** This will array pw in 20 increments starting at  $32.6\mu\text{s}$  with a step size of  $0.2\mu\text{s}$ . (i.e.,  $\text{pw}=32.6,32.8,33.0,33.2,\dots$  to  $36.2$ ). If you are unsure of what to enter as an array, start with general array with fewer steps and larger increments, like **pw=28, 30, 32, 34, 36, 38**.
  - Type **pw[1]=8.65** (or whatever the value was when you queried the pw90 above)
  - To display the arrayed parameters type **da**. A listing will appear in the Text Window.
- Type **au** (submit the experiment to acquisition and process data)
- Type **wft(1)**, phase the spectrum (**aph**), then type **vsadj**
- When the experiment finishes, process the data by typing **wft dssh** (weight and fourier transform data, display stacked spectra horizontally)
- On the screen, (with the exception of the first spectrum) you should see a series of spectra that start negative, pass through a null, and then become increasingly positive. Estimate the point where the signal goes from negative values through zero then become positive. This is the location of your  $360^\circ$  pulse.
- Locate the array value that best describes the 'zero point'. For example:
  - Let's assume the 'zero point' ( $360^\circ$  pulse width) corresponds to  $\sim 34.9\mu\text{s}$ .
  - Type **pw90=34.9/4** ( $8.725\mu\text{s}$ ). Your pw90 for this sample is  $8.725\mu\text{s}$ .
- If you do not see a clear null, you may need to run your array again, adjusting the array's increments and/or step size.
- Similar procedures may be utilized to determine  $90^\circ$  pulses for other nuclei. For example,  $^{13}\text{C}$   $90^\circ$  pulses need to be determined for HMQC experiments. For this, the determination may require a longer time since  $\text{nt}=1$  may be inadequate for obtaining sufficient S/N.

