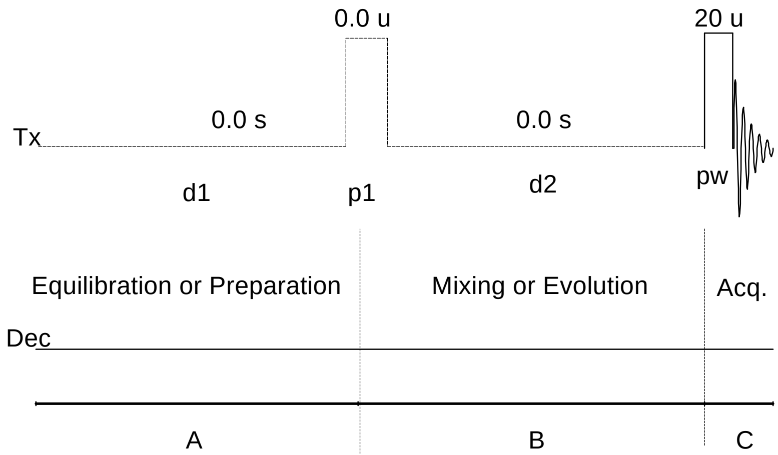
I. The Standard Varian s2pul Pulse Sequence.

The Varian software on both the Gemini and Unity systems uses a simple, but extremely flexible standard pulse sequence, called **s2pul**. Although literally hundreds of other pulse sequences are available, the simple, basic **s2pul** can be used to carry out routine nmr measurements, obtain quantitative nmr spectra, perform homonuclear and heteronuclear decoupling, NOE and solvent suppression experiments. One can also use the **s2pul** sequence to perform relaxation and kinetic measurements.

A diagram of the **s2pul** sequence is shown below:



In the standard 2 pulse sequence, you can define two different delays (**d1** and **d2**), and two different pulses (the observation pulse **pw** and a second pulse **p1**). The delays and pulses can be used to define three time periods. The **d1** delay is used in period **A** to allow the spins to return to equilibrium and prepare the sample for subsequent pulses. The pulse **p1** and the delay **d2** occur during period **B** and can be used to create mixing of magnetization and allow for evolution of spin-coupling information. During period **C**, the signal is acquired after the observation pulse **pw.**

The pulses and delays in the **s2pul** sequence are carried out using a transmitter tuned to the observe frequency of the nucleus of interest. A second transmitter, called the decoupler, is available and can be used to perform simultaneous broad-band or selective excitation of the nucleus being observed. The decoupler can be used to irradiate ^{1}H while observing ^{13}C (on the Gemini), or ^{1}H while observing any other nucleus (on the Unity 500 and UnityPlus 400). On the Varian systems, the decoupler can easily be turned on or off during any of the three periods A, B, and C. The decoupler can also be set to perform selective excitation during one period and then change to broad-band excitation during a different period.

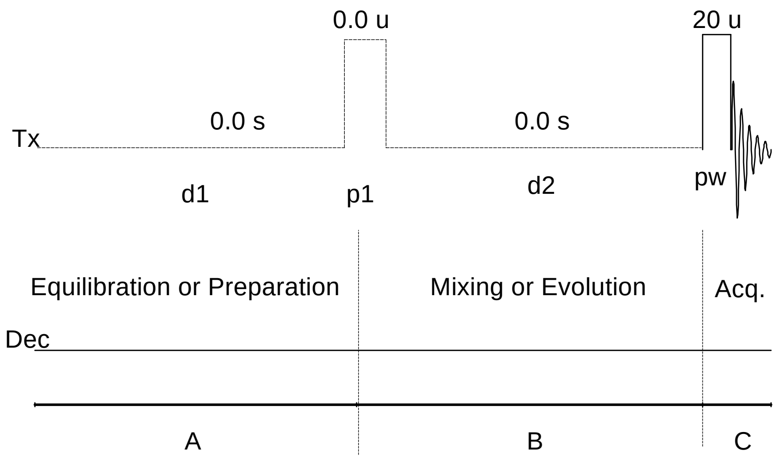
The "status" of the decoupler is controlled by the parameters **dm** (decoupler mode) and **dmm** (decoupler modulation method). During each period A, B, and C, **dm** can be **n** (decoupler off) or **y** (decoupler on). For example, to turn the decoupler on during equilibration and acquisition, or periods A and C, **dm** should be set to **dm='yny'**. **dm='nnn'** would turn the decoupler off during all three periods. **dm='yyy'** would leave the decoupler on at all times. Similarly, the decoupler modulation method can be set to continuous wave (cw or selective irradiation) in each period by setting **dmm='ccc'.** Waltz-modulation (broad-band) excitation is set using **dm='www'** Any combination of cw (**'c'**) and broad-band waltz (**'w'**) decoupling is allowed during each status period. For example, one could selectively excite a single frequency during the equilibration and mixing periods and then switch to broad-band decoupling using **dmm='ccw'** and **dm='yyy'**. Also note that **d1, p1** and **d2** can be zero, enabling the user to eliminate periods A and B from the pulse sequence program.

decoupler mode	**dm='nnn'** to **'yyy'**	**dm** can be **n** or **y** during equilibration, mixing and acquisition. The decoupler will be off if **dm** = **'n'** and on if **dm = 'y'** during the period.
decoupler modulation method	**dmm='ccc'** to **'www'**	**dmm** switches the decoupler irradiation from continuous wave (**'c'**) to broad-band waltz modulation (**'w'**) during each of the three periods.

II. Some Possible Experiments using s2pul.

The combined use of **dmm, dm, d1, p1** and **d2** in a single pulse sequence enables a surprising number of useful nmr experiments to be easily performed, as shown in the following sections. T_1, T kinetics, and NOE measurements are covered in detail in separate manuals.

A. Simple ¹H, ¹³C and other X-nucleus Spectra

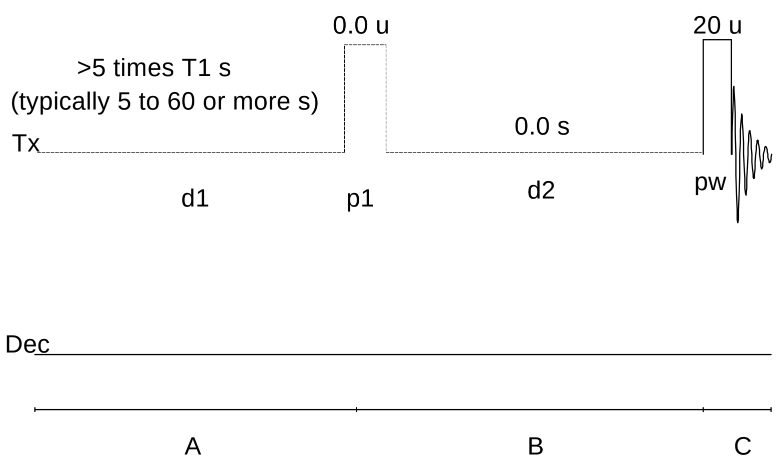


The most basic NMR acquisition requires a single pulse, followed by acquisition of the spectra. The spectra are acquired without decoupling. The complete sequence is repeated **nt** times, until the desired signal to noise is obtained.

d1=0
d2=0
p1=0
pw=90 degrees or less
dm='nnn'
dmm='ccc' (not important, since the decoupler is off at all times)

The transmitter **Tx** is set to the frequency of the nucleus of interest, e.g., ^{1}H, ^{13}C, ^{31}P, etc.

B. Quantitative ¹H Spectra and Kinetics Measurements

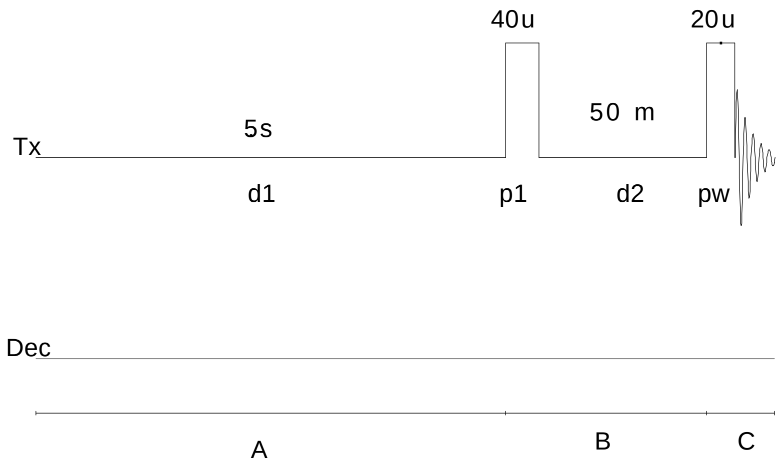


Quantitative ^{1}H spectra also require a single, 90° pulse and a delay **d1** that is allows the magnetization to return to its original equilibrium state. The spectra are acquired without decoupling. The complete sequence is repeated **nt** times, until the desired signal to noise is obtained.

d1=5 to 60 sec or more (>5 times T_1)
d2=0
p1=0
pw=90 degrees (check pw90!)
dm='nnn'
dmm='ccc' (not important, since the decoupler is off at all times)

The simple quantitative sequence can be used to perform kinetics measurements as well. Simply make an array of experiments. For example, 8 spectra of sixteen transients could be acquired by setting **nt=16,16,16,16,16,16,16,16** (type **NT(1)=16,16,16,16,16,16,16,16** on the Gemini-300). One could also make an array of spectra, but wait a fixed time between acquiring successive spectra using a **p**re-**a**cquisition **d**elay, **pad**. For example, 5 minutes could be left between successive spectra by typing **nt=1**, and **pad**=0,300,300,300, etc. (the **pad**=0 would immediately acquire the fid).

C. Inversion Recovery T_1 Measurements

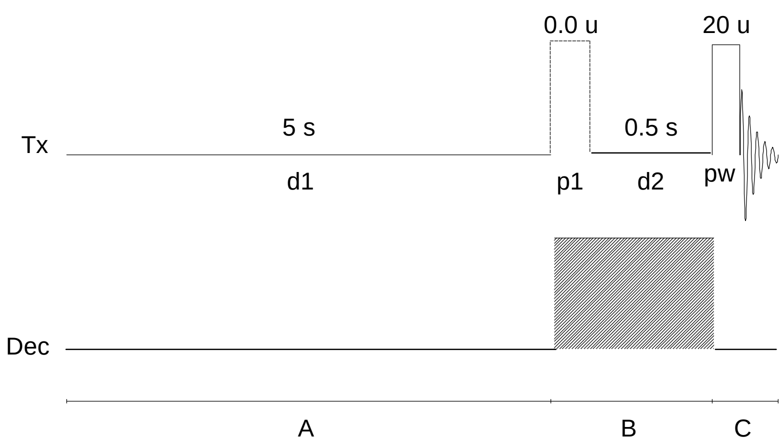


T_1 measurements require a long (5x T_1) delay of time **d1**, followed by a 180° *inversion* pulse **p1**. The magnetization is allowed to *recover* for a time **d2**. The residual magnetization is then examines by a quantitative 90° pulse **pw.** The sequence is repeated for **nt** transients at various values of **d2**.

d1=5 x T_1
p1=180°
d2=array from ca. 0.01 x T_1 to 3-5 x T_1
pw=90°

In the inversion-recovery T_1 measurement, **d2** is arrayed to cover the exponential relaxation of the magnetization. The command **dot1** will generate an appropriate array of **d2** delays, based upon guesses of the shortest and longest relaxations and the overall desired experiment time. **dot1** will also set **p1, d1** and **pw** to the proper values.

D. Homonuclear NOE Measurement



In the homonuclear NOE experiment a spectrum is acquired with single-frequency (selective) CW irradiation of one resonance. This spectrum is subtracted from a second spectra acquired without irradiation of the peak.

d1=5 x T_1 (typically 5-20 s)
d2=1 to 5 x T_1
pw=90° or less
dm='nyn'
dmm='ccc'

The homonuclear NOE measurement requires an array of decoupler frequencies. The first spectrum is acquired with the decoupler off-resonance from any peaks. Successive spectra are acquired with irradiation of a single peak for a time **d2**. The decoupler is turned off and the transient is acquired after the pulse **pw.** The spin-system is allowed to return to equilibrium during the time

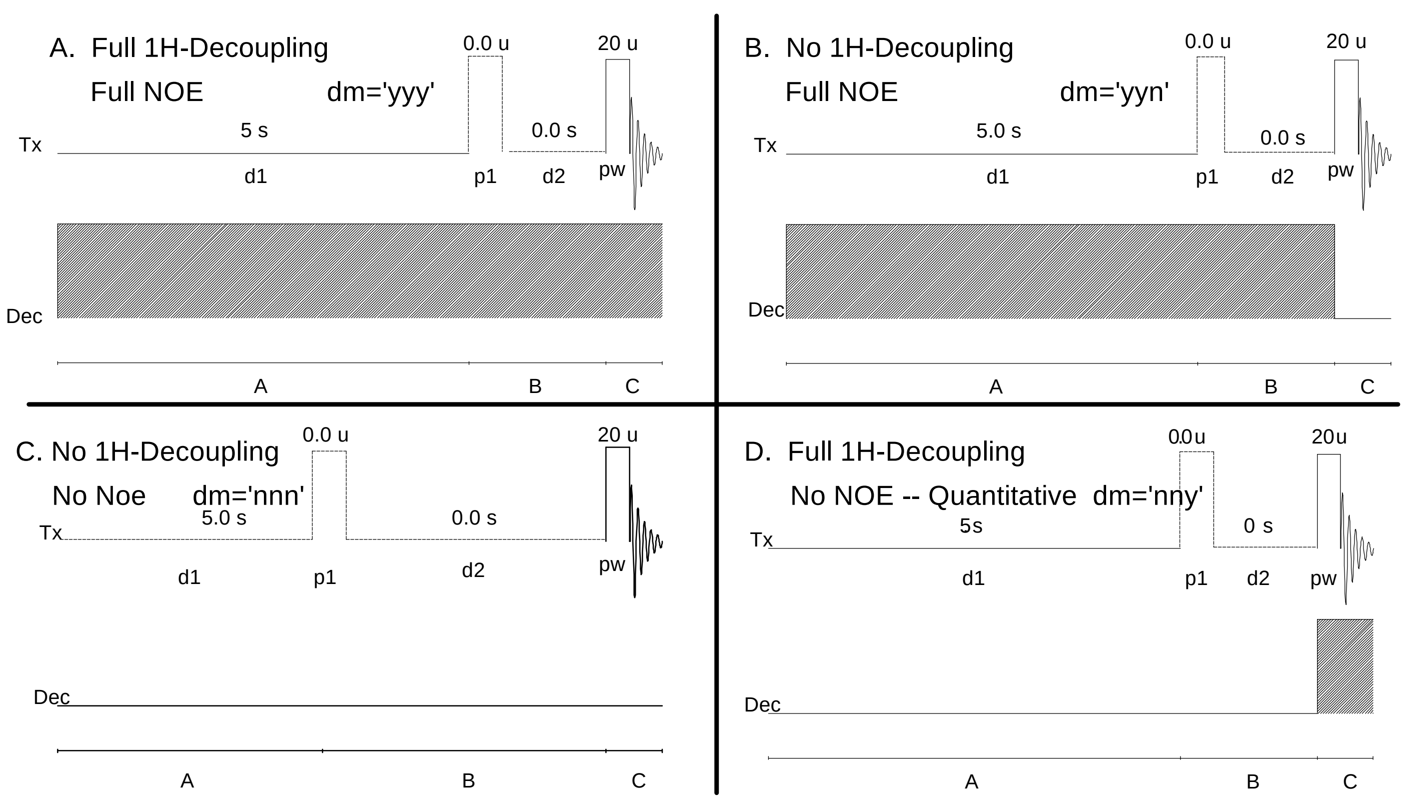
d1. Setup, acquisition, and processing of the homonuclear NOE measurement is covered in detail in a separate handout.

E. The Four Basic Carbon (x-nucleus) Experiments

Carbon is spin one-half and strongly couples to directly attached protons (J = 80-160 Hz). Even protons removed by several bonds lengths can show couplings to carbon on the order of 1 to 10 Hz . Hence, most carbon spectra are obtained with ^{1}H decoupling, in order to remove splitting from protons. ^{1}H decoupling will usually lead to a significant increase in the signal intensities of the carbon signals as well, from NOE enhancement. The increased signal strength caused by NOE is usually desired, due to the low sensitivity of most carbon experiments.

A disadvantage of simple ^{1}H decoupling is that it is impossible to make quantitive measurements from these spectra without first knowing the strength of the NOE values for each resonance. In addition, it is sometimes desirable to observe all the carbon couplings. Unfortunately, this experiment suffers from extremely low sensitivity: the peaks are split into multiplets and there is no NOE enhancement. We can take advantage of the ability to gate the decoupler on and off at various periods to assist with these different meausurements.

Four basic ways of acquiring ^{13}C (or other x-nuclei as well) can be generated from a combination of decoupling and NOE effects. Spectra can be obtained with decoupling and NOE; no decoupling and full NOE; decoupling and no NOE, or no decoupling with no NOE. These four experiments are shown in the following figure. These four experiments take advantage of the ability to easily turn on or off the decoupler at different periods in the NMR experiment, using the **dm** command.



In example A., the broad-band decoupling is used throughout the complete experiment to give a fully decoupled carbon spectrum with full NOE (**dm='yyy', dmm='www')**. The delay **d1** is optional and may be used in conjunction with the pulse width **pw** to optimize sensitivity. The NOE enhancement is retained in example B, however, the spectra is acquired without proton decoupling, i.e., the carbons will all show couplings or splittings from the attached protons (**dm='yyn', dmm='wwc'**). The delay **d1** is required in this example and should be 1-5 x T_1 of the protons in order to build up the NOE enhancement. The pulse sequence in example C., (**dm='nnn', dmm='ccc'**) would yield a fully *coupled* carbon spectrum with no NOE enhancement. If **d1** was set to 5 X T_1 for ***carbons***, this experiment would give quantitative integrals for the resulting carbon multiplets. Example C. is the least sensitive of the four experiments and can easily take 10 to 100 times longer to acquire than the decoupled, NOE enhanced spectrum (example A). Example D. shows how to acquire a quantitative carbon experiment (**pw=90°, dm='nny', dmm='ccw', d1=5 x T_1**). The delay **d1** is set to 5 times the T_1 of the ^{13}C atoms of interest. The pulse width **pw** is set to 90° and the decoupler is turned on ***only*** during acquisition to prevent buildup of NOE enhancement.

Examples of these four ^{13}C experiments, performed on the Gemini-300 using a solution of 30% Menthol in $CDCl_3$ (**nt=1, d1=10)** are shown below.