Basic Practical NMR Concepts:

A Guide for the Modern Laboratory

Description:

This handout is designed to furnish you with a basic understanding of Nuclear Magnetic Resonance (NMR) Spectroscopy as it pertains to running the instrument. The concepts implicit and fundamental to the operation of a modern NMR spectrometer, with generic illustrations where appropriate, will be described. It can be read without having to be in front of the spectrometer itself. Some basic understanding of NMR spectroscopy is assumed. An excellent introduction to NMR can be found on the web at http://www.cis.rit.edu/htbooks/nmr/inside.htm.

IMPORTANT: There is a short written test at the end of this handout, which must be taken in order to obtain an NMR account.

This handout was prepared by Dr. Daniel Holmes of Michigan State University using the NMR Basic Concepts handout from the University of Illinois's NMR service facility, under the direction of Dr. Vera V. Mainz. Her generous contribution is gratefully acknowledged. February 2004.

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Introduction

Nuclear Magnetic Resonance (NMR) is a powerful non-selective, nondestructive analytical tool that enables you to ascertain molecular structure including relative configuration, relative and absolute concentrations, and even intermolecular interactions of an analyte. Once challenging and specialized NMR techniques have become routine with little more than a push of a button to obtain highly complex data. NMR is indeed an indispensable tool for the modern scientist. Care must be taken, however, when using such 'black box' approaches. While the standard parameters used in the set-up macros for experiments might be adequate for one sample, they may be wrong for another. A single incorrectly set parameter can mean the difference between getting an accurate, realistic spectrum and getting a meaningless result. A basic understanding of a few key aspects of NMR spectroscopy can ensure that you obtain the best results possible. This guide is intended to highlight the most pertinent aspects of practical NMR spectroscopy.

"Modern pulse NMR is performed exclusively in the Fourier Transform (FT) mode. Of course it is useful to appreciate the advantages of the transform, and particularly the spectacular results that can be achieved by applying it in more than one dimension, but it is also essential to understand the limitations imposed by digital signal analysis. The sampling of signals, and their manipulation by computer, often limit the accuracy of various measurements of frequency and amplitude, and may even prevent the detection of signals altogether in certain cases. These are not difficult matters to understand, but they often seem rather abstract to newcomers to FT NMR. Even if you do not intend to operate a spectrometer, it is irresponsible not to acquire some familiarity with the interaction between parameters such as acquisition time and resolution, or

repetition rate, relaxation times and signal intensity. Many errors in the use of modern NMR arise because of a lack of understanding of its limitations."

From A.E. Derome, Modem NMR Techniques for Chemistry Research (1987)

Basics of FT NMR- Six Critical Parameters

This section will give you enough information about FT-NMR experiments to avoid the most common errors. We will cover the most important parameters that affect any spectrum you may collect using an FT-NMR spectrometer. These are:

1. Spectrometer Frequency [sfrq]
2. Pulse Width [pw]
3. Acquisition Time [at]
4. Number of Points [np]
5. Sweep (Spectral) Width [sw]
6. Recycle Delay [d1]

[The letters in square brackets following the parameter represent the mnemonic used on all Varian/Agilent spectrometers. The parameters are discussed in more detail below.]

The most basic and common pulse sequence you will encounter is the '1PULSE' FT-NMR experiment, which is the sequence used for routine ^{1}H and, with the addition of a decoupling field, $^{13}C\{^{1}H\}$ acquisitions. It can be represented as shown in Figure 1. In a typical NMR acquisition, this pulse sequence will be repeated many times in order to improve signal-to-noise (S/N), which increases as the square root of the number of scans (nt). The user can independently set each of the parameters shown in Figure 1. Knowledge of their purpose and function will help you obtain quality NMR spectra. On Varian/Agilent spectrometers, you can view the current pulse sequence by typing 'dps'.

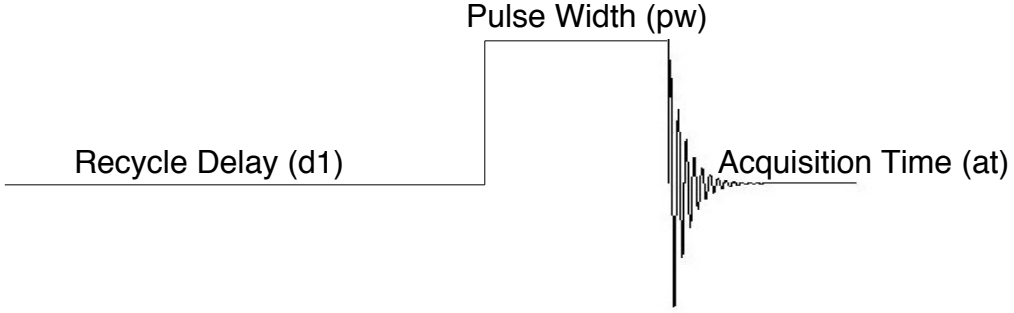


Figure 1. Schematic representation of one cycle of a simple '1PULSE' pulse sequence.

1. **Spectrometer Frequency [sfrq]:**

It is called a "1PULSE" experiment because one radio frequency pulse (pw) is applied per cycle. The radio frequency pulse (usually in microseconds) excites the nuclei, which then relax during the acquisition time, giving an NMR signal due to an oscillating voltage induced by the precession of the nuclear spin in the X-Y plane. This results in the observed exponentially decaying sine wave. This decaying sine wave is termed free-induction decay (FID). The radio pulse has a characteristic frequency, called the spectrometer frequency (sfrq), which is dependent upon the nucleus you wish to observe and the magnetic field strength of the spectrometer. NMR spectrometers are generally named for the frequency at which protons will resonate. Thus, a 500 spectrometer will cause protons to resonate at approximately 500 MHz. The spectrometer frequency defines the center of the NMR spectrum you acquire. A 500 MHz NMR Spectrometer has a field strength of 11.74 Tesla. The relationship between a nucleus' frequency and spectrometer field strength is given by:

$$\nu = \frac{-\gamma B_o}{2\pi} Hz$$

, where γ is the gyromagnetic ratio (26.7522128 x 10^7 rad $T^{-1}s^{-1}$ for proton and 6.728 x 10^7 rad $T^{-1}s^{-1}$ for carbon) and B_0 is the field strength of the magnet expressed in Tesla.

The RF pulses used in FT-NMR need to have an effective excitation field that excites all nuclei of interest equally (calculated from 1/[4*90° pulse(sec)]). For ^{1}H on a 500 MHz spectrum, this equates to ~5000 Hz. A typical 90° pulse is around 10 μs, which gives a RF field of 25000 Hz. This easily covers the chemical shift range seen in typical NMR experiments (~10 ppm for ^{1}H and ~250 ppm for ^{13}C). Shortening the pulse length will result in a larger bandwidth of excitation (~90% even excitation is achieved with 1/2pw). A longer pulse will have a smaller RF field and can be used for frequency selective excitation or saturation. For example, a 10 μs pulse will give efficient excitation of 50,000 Hz; whereas, a 1000 μs pulse will excite over a bandwidth of 500

Hz.

2. **Pulse width [pw]:**

Prior to applying a radio frequency pulse, a slight majority of nuclear spins are aligned parallel to the static magnetic field (B_0) (at 500 MHz, this equates to about 0.008%). The axis of alignment is typically designated the Z-axis and the bulk magnetization is shown as a bold arrow (Figure 2, left side). Application of a short radio frequency pulse at the appropriate frequency will rotate the magnetization by a specific angle [$\theta=360(\lambda/2\pi)B_1t_p$ degrees, where $(\lambda/2\pi)B_1$ is the RF field strength and t_p is the time of the pulse]. Pulses are generally described by this angle of rotation (also called flip angle). The amount of rotation is dependent on the power (tpwr) and width of the pulse in microseconds (pw). Maximum signal is obtained with a 90º pulse. Thus, a 90º pulse width is the amount of time the pulse of energy is applied to the particular sample in order to flip all the spins into the X-Y plane, i.e., the condition shown in Figure 2A. The 90º pulse width for proton NMR experiments is set to about 8-13 μs on most instruments. The approximate field width of excitation is given by the formula, RF_{field} =1/(4*90ºpulse). Thus, for a 8 μs, the field is 1/(4*0.000008) = 31250 Hz, which is ample for the typical range of proton resonances in organic samples (at 500 MHz the proton range is about 5000 to 7000 Hz). Reducing the length of the pulse width will increase the bandwidth of excitation. ~70% excitation is achieved, with attenuation at the edges, as the reciprocal of the pulse width (e.g. a 4 μs pulse gives 70% excitation over 250 kHz). The pulse width is entered in microseconds by typing pw=desired value. The exact value is dependent upon the sample (nucleus, solvent, etc.) as well as the instrument (probe, etc.). Methods for measuring the pulse width will be discussed in another handout and are, for the most part, only required for advanced experiments. For routine experiments, most users use a 45º pulse for their data collection (Figure 2B). The reasons for this are discussed under recycle delay.

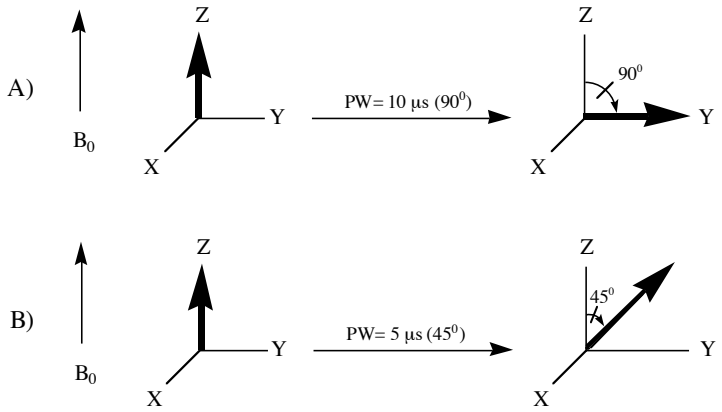


Figure 2. The average nuclear spin magnetization (bold arrow) for an NMR sample placed in a magnetic field aligned along the Z-axis before and after application of a pulse.

3. **Acquisition time (*at*):**

Thus far, we have sent a pulse through the sample and flipped the magnetization by a specific angle. The nuclear spins are no longer at equilibrium and will return to equilibrium along the Z-axis. In Figure 1, the decaying sine wave represents this process of Free Induction Decay (FID), which is a plot of emitted radio intensity as a function of time. The time you specify to acquire the FID is called the **acquisition time** and is set by the parameter '*at'*. A natural inclination might be to increase the acquisition time to maximize the amount of signal that is acquired. Increasing the acquisition time is advantageous up to a point, but will be detrimental if extended too far. Care and forethought should be taken when adjusting '*at'*: too long and you will acquire noise unnecessarily; too short and extraneous wiggles will occur at the base of the peaks (read zero-filling section for more information).

4. **Number of points (np):**

The tiny analog signal emitted from the sample (in microvolts) is amplified, mixed, filtered, and attenuated prior to digitization, which is required for further computer processing. The ADC (analog-to-digital converter) converts the analog FID

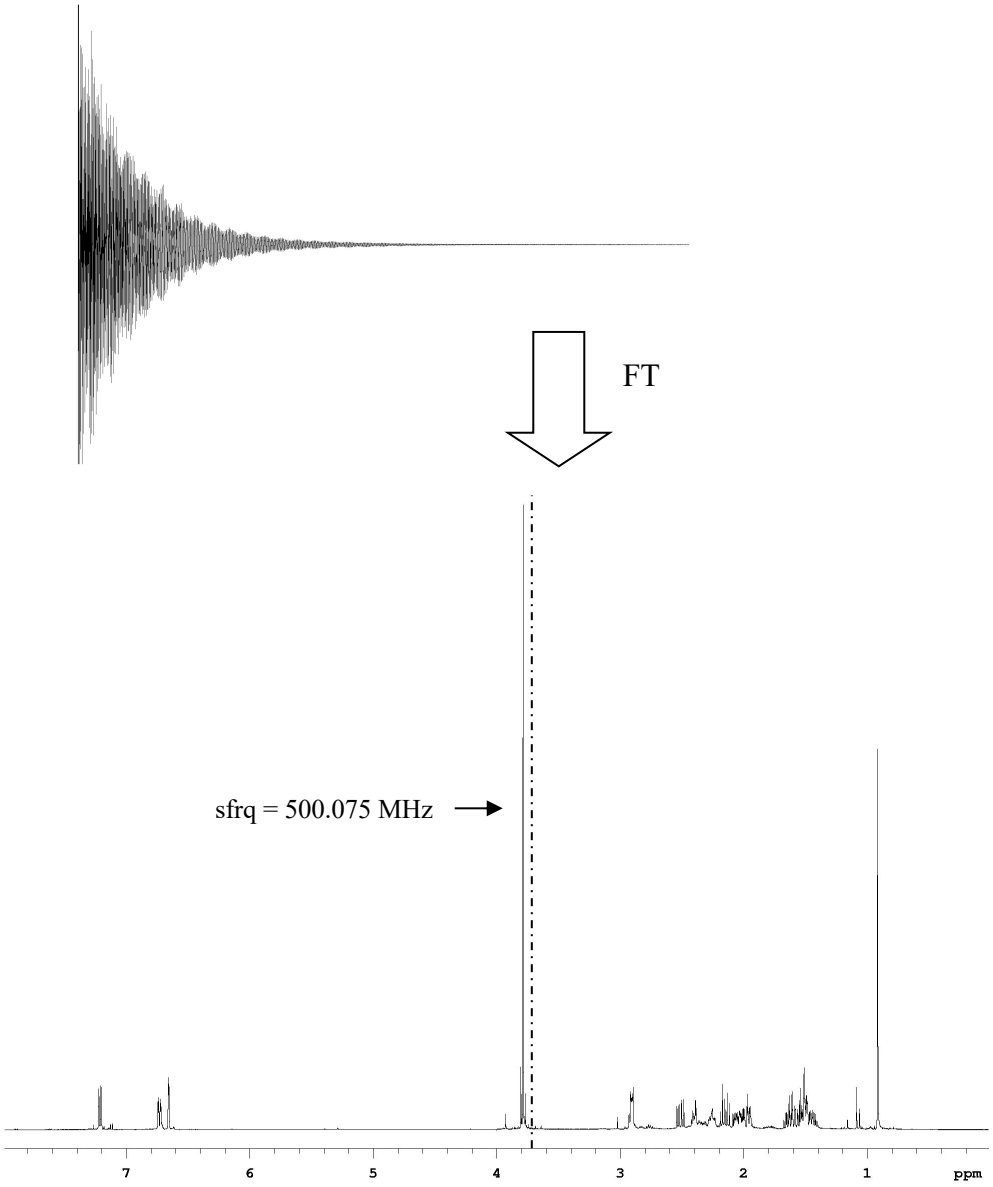


Figure 3. Fourier transform of the FID for estrone acquired at 500 MHz. Note: the spectrometer frequency you use, in general, will not be exactly 500 MHz.

into a series of points along the FID curve. This is the number of points (np). In general, the more points used to define the FID, the higher resolution. The number of points (np), sweep width (sw), and acquisition time (at) are interrelated. Changing one of these parameters will affect the other two (see below).

5. **Sweep Width (sw):**

While the FID contains all the requisite information we desire, it is in a form that we cannot readily interpret. Fourier transforming the FID (commonly referred to as FT or FFT for Fast Fourier Transform) will produce a spectrum with the familiar intensity as a function of frequency, as shown in Figure 3. The frequency domain spectrum has two important parameters associated with it: the spectrometer frequency (sfrq), discussed earlier, and the spectral width or sweep width (referred to as sw- see Figure 4). It is important to remember that the spectral width **in ppm** is independent of the spectrometer operating frequency; however, since the number of Hz per ppm is dependent on the spectrometer operating frequency, the spectral width **in Hz** will change depending upon the spectrometer used and the nucleus observed. For example, at a spectrometer frequency of 300 MHz, a spectral width of approximately 3000 Hz is needed to 'scan' 10 ppm in ^{1}H, since each ppm contains 300 Hz (10 ppm x 300 Hz/ppm = 3000 Hz). At a spectrometer frequency of 500 MHz, a spectral width of approximately 5000 Hz is needed to 'scan' 10 ppm (10 ppm x 500 Hz/ppm). Carbon frequencies are ¼ that of the corresponding proton frequency. Thus, on a 300 MHz spectrometer, carbons resonate at 75 MHz and 10 ppm is 750 Hz (on a 500 MHz spectrometer, 13C resonate at 125 MHz and 10 ppm is 1250 Hz).

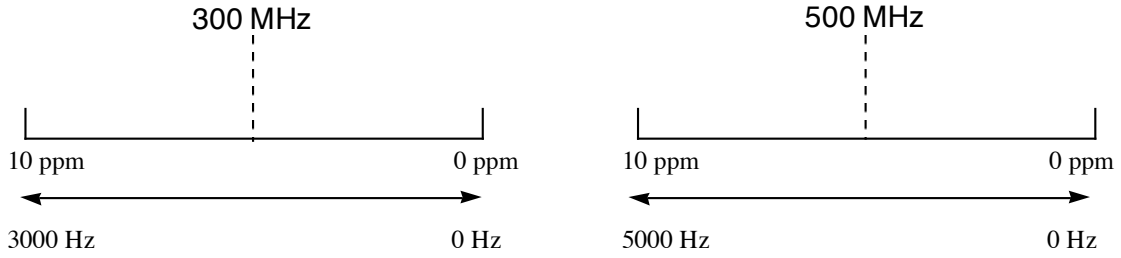


Figure 4. The spectral width in ppm and Hertz at different spectrometer frequencies for Proton. Note the difference in the spectral width in Hertz for the two spectrometers.

The sweep width (sw), number of points (np), and the acquisition time (at) are related by the following equations:

$$at = \frac{np}{2sw} \qquad (1)$$

and

$$res = \frac{1}{at} = \frac{2sw}{np} \qquad (2)$$

where ‘res’ is the digital resolution of the spectrum. The digital resolution is in units of Hz/point, and the rule-of-thumb is that the digital resolution (in Hertz) should be less than one half the peak width at half-height. This ensures that each peak is described by at least 3 points. For example, if your peak width at half-height is 0.5 Hz, the digital resolution should be less than 0.25 Hz. Therefore, if your spectrometer frequency is 500 MHz, your total spectral width is 5000 Hz (10 ppm) and your required digital resolution (Res) is 0.25 Hz/point, rearranging equation 2 gives you the minimum number of points required for adequate digital resolution:

$$np = \frac{2sw}{res} = 40{,}000 \text{ points} \qquad (3)$$

Since the number of points must be a power of 2, the closest larger power of 2 would automatically be used, which, in this case, is 65,536 points. The spectral width, number of points, and acquisition time can be specified when operating the spectrometer, usually by typing the appropriate mnemonic followed by an equals sign and the numeric value (e.g. np=64000). The spectrometer will set the units automatically. Generally, the spectrometer will automatically change the number of points according to equation 2 if the acquisition time or sweep width are changed. If at, np, or sw are changed, the data must be reacquired. An alternative to changing these parameters is to use ‘zero-filling’. This is described in the section titled ‘Zero-Filling’.

You may have noticed that NMR spectroscopists tend to use the terms ‘sweep width’ and ‘scans’ when referring to collecting data. These are historical terms that were only truly appropriate for spectrometers prior to the use of pulsed NMR techniques (FT NMR spectrometers were introduced commercially in 1969). Prior to FT NMR either the magnetic field was swept or the spectral region was scanned with a radio frequency source. The terms ‘high’ (upfield: lower ppm) and ‘low field’ (downfield: higher ppm)

originated from these type of Continuous Wave NMR and are still in use today.

6. **Recycle delay (d1):**

On Varian (Agilent) and Bruker NMR spectrometers, the delay time is named d1 (pronounced dee-one) and appears at the beginning of the pulse sequence (see Figure 1). In practice, this delay should be thought of as coming after the acquisition time. It is an important parameter and plays a vital role in obtaining accurate integration. After the RF pulse, the nuclear spins do not instantly return to equilibrium; rather, they relax according to a time constant called T_1 (T_1 is 1/R, where R is the rate of relaxation. After one T_1, approximately 63% of the magnetization has returned to the Z-axis). T_1's are dependent on many factors including nuclear environment, temperature, and solvent. Carbon T_1's are typically much longer than proton T_1's. Since each nucleus in a molecule is immersed in a different magnetic environment, their T_1's will not be the same. Not allowing enough time for relaxation between pulses will cause varied attenuation of the signals and inaccurate integration (see Integration Section for more details). Normally, when a 90° pulse width is used to excite the spins (Figure 2A), a total time (TT) between pulses of 5x(longest T_1) is necessary in order to have complete relaxation. If a pulse width less than 90° is used, the total time can be proportionally less. This is why the standard pulse width for 1D ^{1}H NMR experiments is 45º.

The total time between scans is given by the following equation, where TT is the total time and d1 is the recycle delay: TT = pw + at + d1.

Since the pulse width is in microseconds while the acquisition time and recycle delay are in seconds, the pulse width can be ignored, leaving us with the equation:

$$TT = at + d1 \qquad (4)$$

The optimum recycle delay can be computed by rearranging the equation to give

$$d1 = TT - at \qquad (5)$$

As an example of the above, if your longest T_1 is 0.600 seconds, then the total time (where TT=5x T_1) must be at least 3 seconds.

Take Home Lesson

These six parameters provide the foundation on which all NMR experiments are built. Appreciation of them will go far in the correct acquisition and interpretation of your NMR spectra, thus, saving precious time and effort. This not only applies to simple 1PULSE experiments, but also is equally important in 2-D and 3-D NMR spectroscopy.

Applications of FT-NMR: Important Variables

1. **Peak Width at Half Height ($LW_{1/2}$).**

The purpose of this section is to acquaint you with proper peak shape and the problems that are caused by improper shimming.

NMR peaks have a shape that is called Lorentzian. A Lorentzian line can be expressed mathematically and has three parameters: amplitude [A], width at half-height in Hz [$LW_{1/2}$] and position, in Hz [X_0]. An example of a Lorentzian line with $LW_{1/2}$ = 0.25 Hz is shown below, in Figure 5. Also, NMR spectra are typically displayed as an absorption spectrum (signal is as shown below as opposed to dispersive, which has the signal dispersed equally above and below the baseline, see Phasing section on next page).

$$Y = \frac{A(LW_{1/2})^2}{(LW_{1/2})^2 + 4(X_0 - X)^2}$$

A = Amplitude

$LW_{1/2}$ = Peak width at half height, in Hz

X_0 = Peak position, in Hz

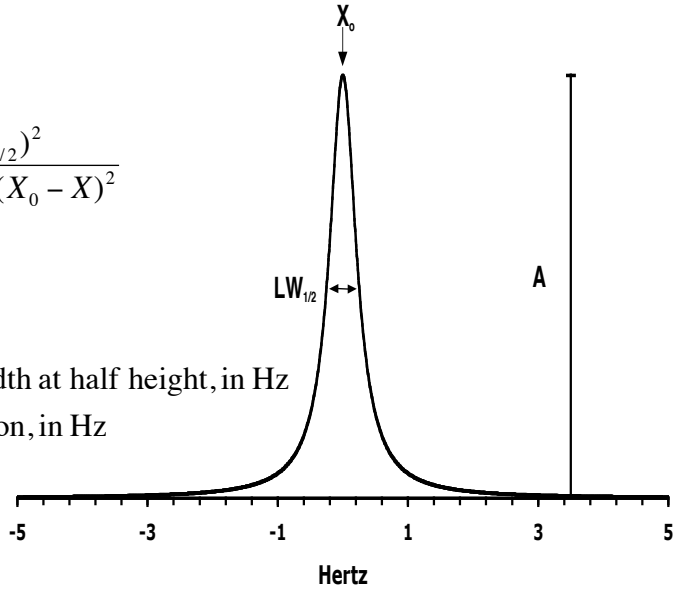


Figure 5. Absorptive Lorentzian line with $LW_{1/2}$=0.25 Hz.

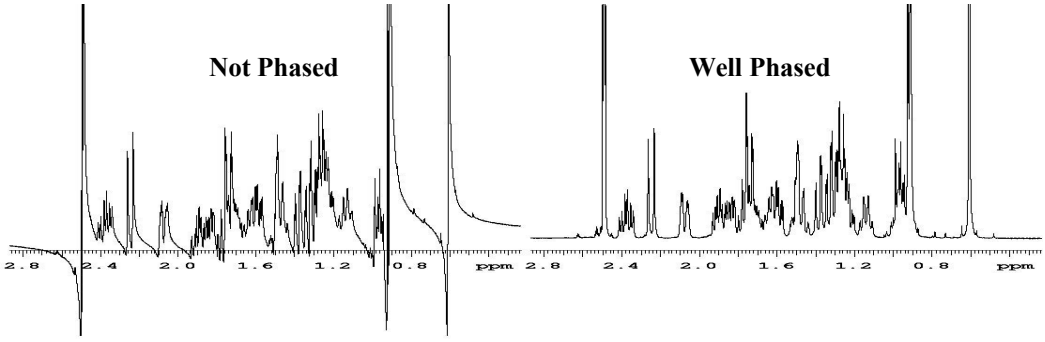
The minimum obtainable peak width at half-height is directly related to the resolution of an instrument; i.e., how close two peaks can be and still be distinguishable. Resolution is usually measured using *o*-dichlorobenzene, which has very narrow lines in its ^{1}H NMR spectrum. The manufacturers' resolution specification is usually 0.20 Hz.

Manufacturers of NMR instruments, however, have traditionally separated the resolution specification from the lineshape specification. Line shapes for ^{1}H NMR spectra are usually specified using $CHCl_3$ and the specifications are stated in terms of the peak width at half-height, 0.55%, and 0.11 % height of the $CHCl_3$ peak. The latter two

percentages are chosen because they are the height of the ^{13}C satellites of the $CHCl_3$ line and one-fifth this height. These values are meaningful only when compared with the half-height width. From the mathematical equation for a Lorentzian line (Figure 5), the line width at 0.55% height is calculated to be 13.5 times $LW_{1/2}$, while the line width at 0.11 % height is calculated to be 30 times the $LW_{1/2}$. So, if the peak width at half-height is 0.30 Hz, the calculated values are 4.0 Hz at 0.55% and 9.0 Hz at 0.11 %. For comparison, the manufacturer's specifications are 10-15 Hz and 20-30 Hz at 0.55% height and 0.11 % height, respectively. These values are larger than the theoretical values because the line widths at 0.55% and 0.11 % height are very sensitive to shimming. Other factors that influence lineshape include the quality of the NMR tube, sample spinning, sample concentration, dissolved oxygen, and paramagnetic impurities. The latter three will lead to an overall broadening of the lines. In general, if your solvent peak has a $LW_{1/2}$ less than 1Hz, it is properly shimmed.

Phasing:

Due, in part, to delays in the pulse sequence between excitation and reception and to frequency offset errors; acquired spectra will have a mix of absorptive and dispersive signals. Your spectrum's peaks will not look like the lorentzian in Figure 5, but may have some portion that is displaced below the baseline. As a user, you will have to correct the spectrum by adjusting the 'phase' of the spectrum. The 'Software Practice' handout will help you with phasing spectra. For now, it is only important to know that phasing the spectrum is routine and involves correcting two parameters: zero-order phase, which is frequency independent; and first-order phase, which is frequency dependent. Correcting the phase is as simple as typing a command or doing a little bit of 'click-and-drag' mouse work. Below is an example of a 'poorly' phased spectrum at left along with the correct spectrum (i.e. purely absorptive peaks).



Shimming:

The term ‘shimming a magnet’ is a piece of NMR jargon that harkens back to the early days of NMR spectroscopy. Originally, permanent magnets were used to provide the external magnetic field. To obtain the most homogenous field across the sample, the pole faces of the magnet had to be perfectly aligned, and to accomplish this, small pieces of wood, or ‘shims’, were hammered into the magnet support, so as to physically move the poles relative to each other. Luckily, nowadays you will not be required to bring hammer and wooden shims to the spectrometer. Shimming is accomplished by changing the applied current for a set of coils surrounding the probe. This applied current will create small magnetic fields in the region of your sample that will either enhance or oppose the static magnetic field. Your goal will be to adjust these coil fields by a series of mouse clicks to obtain the most homogeneous magnetic field across your sample, which is usually observed as an increase in the lock signal. Most modern spectrometer will perform this automatically, but it is still essential to understand the underlying principles.

It is important for you to have a basic understanding of lineshape so you can judge when: (1) your shimming is off, and (2) you need to spend more time shimming your sample. The best way to avoid problems is to establish a procedure, such as the one detailed below.

I. Always load a shim library when you sit down at the instrument. You should never assume the previous user left the instrument with a standard shim library loaded. Without reloading standard shims, you will have to start where the last person stopped - and that might include someone who shimmed for a short sample, a bad tube, a viscous sample, etc.

II. Be aware of lock parameters, especially if you only shim on the lock display. Establish lock transmitter power and gain levels that work for most of your samples. If you encounter a sample that seems to require an unusually high power or gain setting, there is a problem with your sample and/or the instrument, and shimming on the lock level may be difficult or impossible.

III. Shimming problems are confirmed only if the problem is visible on <u>every</u> peak in your spectrum. If, for example, only one peak is doubled, the

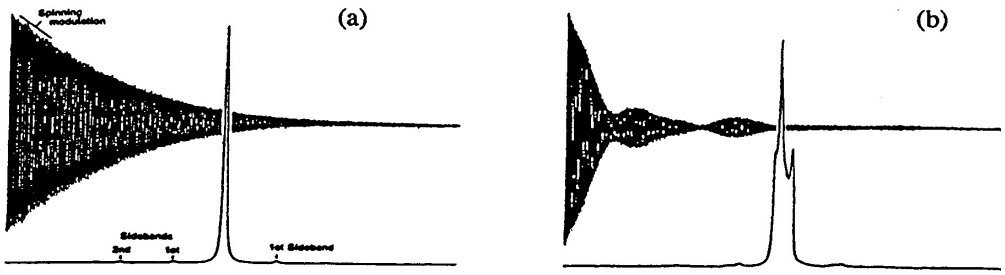
problem is sample related, and can't be shimmed away. Remember, anomalies close to the base of intense single lines may not be visible on less intense peaks unless the vertical scale is increased.

IV. Establish a shimming method. Shimming is an ‘art form’ that requires patience and practice. You should always approach shimming with some method that works for you to give acceptable results. Example: load a shim library; adjust the lock level to a maximum with Z1, then Z2, then Z1, then Z3, and then Z1.

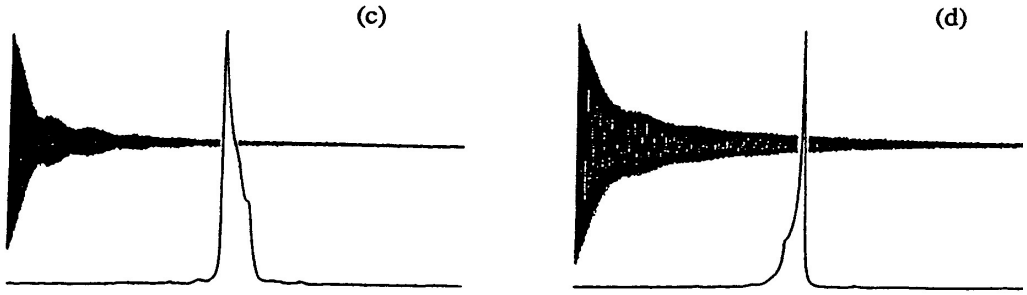
V. Spinning side bands should always be below 2%. If spinning side bands are above 2%, turn off the spinner air, optimize the X and Y shims, then turn the spinner air back on and re-optimize Z1, Z2, and Z3. If this does not solve the problem, consider transferring your sample to another tube.

Knowledge of correct lineshape can help you correct problems such as those shown in Figure 6. Although the peak in Figure 6b may have a line width at half-height that is less than 0.50 Hz, it is obviously poorly shimmed. You should never accept a poorly shimmed lineshape such as is shown in Figure 6b, where a single line is expected.

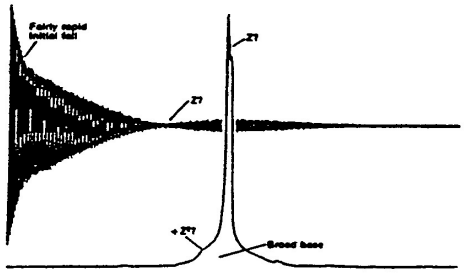
On the pages that follow are some lineshape defects and the shims that should be adjusted to correct the problem. You will also notice that the FID will show the problem as well, but may not be as easy to diagnose. In general, odd-order shims (Zl, Z3, Z5) affect the lineshape symmetrically while even-order shims (Z2, Z4) cause a non-symmetrical lineshape. The higher the order (Z4 is higher order than Z2), the lower (closer to the base of the peaks) the problem is observed.



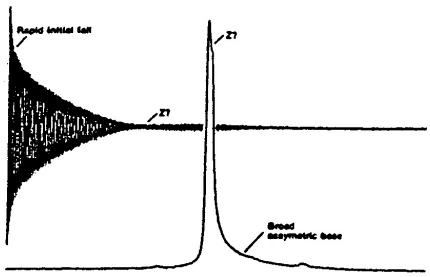
Z1 -- A "good" FID and a spectrum with small spinning sidebands are shown above in (a), but when the "Z" shim is changed (b), a characteristic beat in the FID is obtained, and the spectral line broadens and manifests structure that is an indicator of Z1 inhomogeneity.



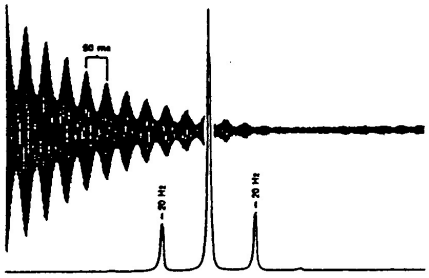
Z2 -- The asymmetric shape of the above peak (c) is typical of a mis-set Z2 shim. Note that the beats in the FID are less pronounced than in the diagram for Z1 (above, figure b) and that the initial descent is steeper. If the Z2 shim is mis-set in the opposite direction, then the asymmetry is reversed (d).



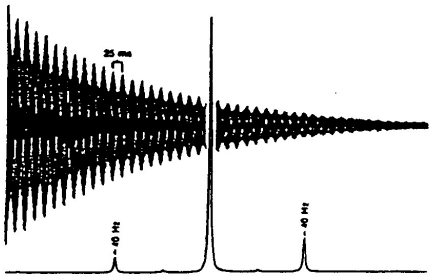
Z3 -- Although the broadened base of this peak is typical of a mis-set Z3 shim as is the rapid initial descent of the FID, there are also hints of +/-Z1 and +Z2 in the lineshape.



Z4 -- The very rapid initial fall of the FID and the pronounced asymmetry are typical of a mis-set Z4 shim. Note that in contrast to the effect of the Z3 shim effect in the previous diagram, there is very little evidence of Z1 or Z2, suggesting that the Z3 and Z4 shims windings are staggered slightly; i.e., their origins differ. Reversal of current in the shim naturally reverses the asymmetry.



X or Y -- The formation of echoes in the FID every 50 ms is clearly visible, and first-order spinning sidebands 20 Hz away from the main spectral line can be seen. First-order spinning sidebands are separated from the main peak by the sample spinning rate (20 Hz).



XY or X^2-Y^2 (R^2) -- Echoes are now formed every 25 ms (see previous diagram), and the spinning sidebands are "second-order," i.e., 40 Hz away from the main line, which is twice the sample spinning rate. The difference in the two sidebands' heights is often seen for mis-set XY or X^2-Y^2 shims.

Figure 6. From G. Chmurny and D. Hoult, "The ancient and honorable art of shimming." *Concepts in Magnetic Resonance*, **1990**, *2*, 131-149.

Shimming Take Home Lesson

The 'art' of shimming resides in the fact that there is no single set of rules that work for every sample, spectrometer, person, or even time of year. Personal experience is the best and, frankly, only way to master shimming. That being said, knowledge of correct line shapes will allow you to decide quickly whether your sample is correctly shimmed. You will have to decide whether the return (a better line shape) is worth the time spent achieving that line shape.

Zero-Filling

As stated earlier, the digital resolution is equal to (acquisition time)$^{-1}$. If you wanted to increase resolution, you might consider increasing the acquisition time (at) to gain more points and, thus, better resolution. This would certainly work, but increasing it too much would sacrifice Signal-to-Noise for the resolution enhancement. The FID has a finite lifetime, which is proportional to the various T_1's for a given molecule. When the acquisition time is significantly longer than the longest T_1, the contribution from noise will be quite large. This combined with the increased overall experimental time

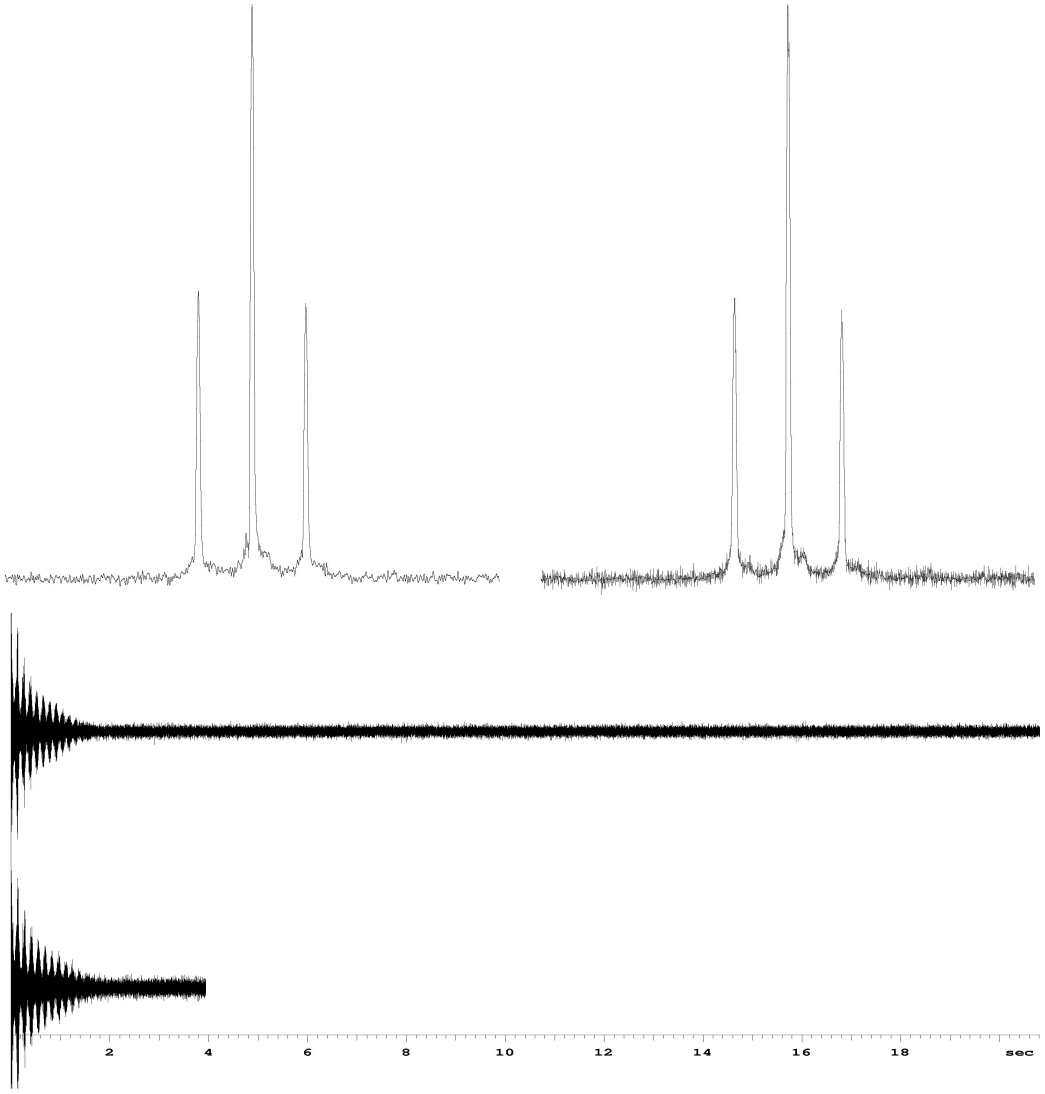


Figure 7. Four scan acquisition of ethylbenzene on an Inova-300. The triplet to the right was acquired with at = 20 seconds, the triplet at left had at = 4 seconds, which gave a S/N twice that of the other acquisition. The FID's are shown below the spectra.

necessary to acquire a given number of scans leads to a significant decrease of S/N. Figure 7 shows the results from two separate acquisitions on the same sample with the same number of scans, but with their acquisitions times differing by a factor of five. The Signal-to-Noise for the 4-second acquisition time (at) is about twice that of the 20-second acquisition time and required a fourth of the time. Note the FID's, which clearly show that the signal decays below the level of noise around two seconds. The additional acquisition time merely adds noise to the spectrum.

Of course, there is a compromise with using a shorter acquisition time: you lose digital resolution. In the spectrum below, there is about a factor-of-four reduction in digital resolution for the shorter acquisition (0.15 Hz/pt vs. 0.04 Hz/pt for at=4 and at=20, respectively). Luckily, there is a means of increasing digital resolution without requiring such long acquisition times. This is accomplished by zero-filling. Zero-filling is simply adding data points with zero intensity to the end of the FID. This will add data points to your FID without adding additional noise. It is important to note, however, that zero-filling does not improve true resolution; it only improves the apparent resolution. This can be very useful because fine coupling may not be visible due to low digital resolution

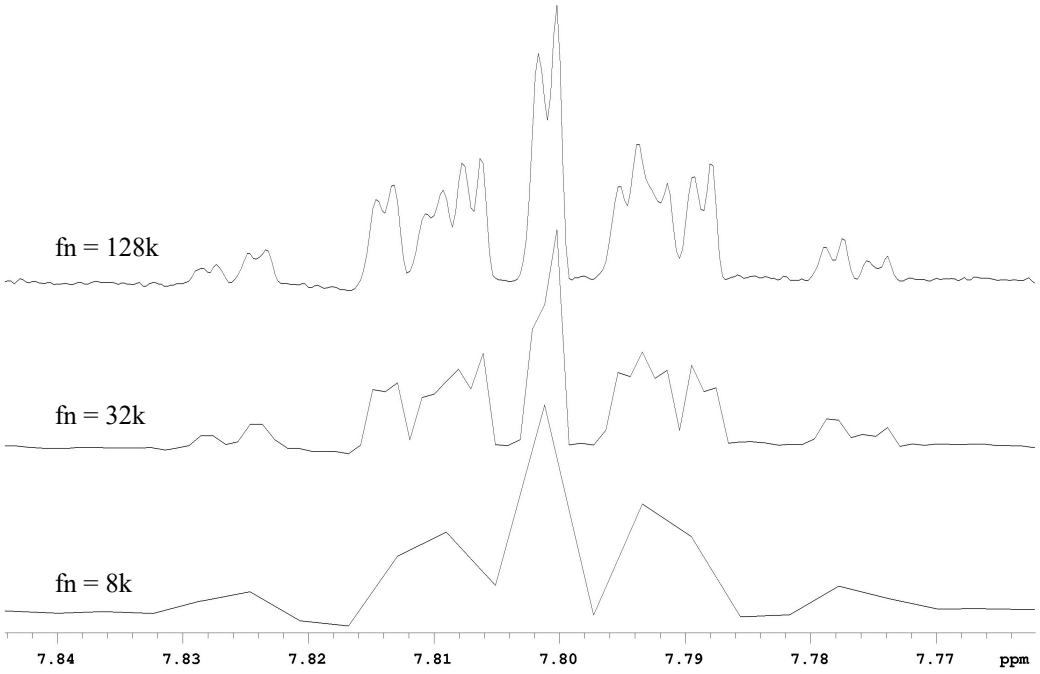


Figure 8. Effect of zero-filling on an aromatic multiplet. Spectrum taken on a Varian UnityPlus 500 Spectrometer.

even though the coupling is resolved in the time domain. Figure 8 shows the effect of zero-filling on a spectrum. At low resolution the fine coupling is not visible, but with adding zeros to the FID, the details of coupling emerge. Varian executes zero-filling through the Fourier number (fn). A Fourier transform will transform fn zeros to the nearest power of two minus np points (e.g. if np=64k and fn=4*np, then the numbers of zeros = $2^{18} - 2^{16}$ or 196608 points. A total of 262144 points will be transformed). In practice, setting fn more than 4 times np is not useful.

One might be tempted by the preceding section to set the acquisition time (at) to a very short value and then use zero-filling to increase the digital resolution. This will lead to spectral artifacts. Figure 9 demonstrates these artifacts for the methyl triplet of ethyl benzene. The spectrum on left has an acquisition time of 1 second and 4 seconds for the one to the right. They both have the same number of points (200k), but clearly the spectrum to the left has artifacts. These artifacts are termed truncation artifacts or, colloquially, *sinc wiggles* [(sin x)/x modulation] and arise from turning off the receiver before the FID has mostly decayed.

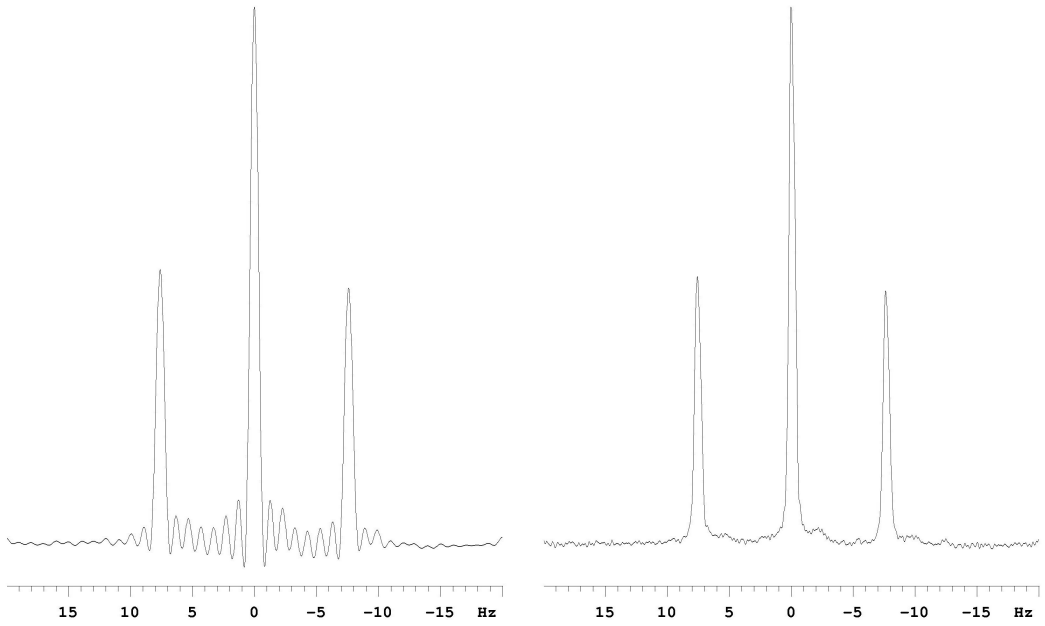


Figure 9. Truncation artifacts or so-called "sinc wiggles" because of too short acquisition time (at=1). Both spectra have 200k points. That to the right has an at=4 seconds with zero-filling to 200k. That to the left has at=1 seconds with zero-filling to 200k. Spectra were taken on a Varian UnityInova 300.

Apodization (Spectral Weighting Functions)

Signal-to-Noise (S/N) is very important for any spectroscopic technique. NMR spectroscopy, unfortunately, suffers from low S/N. Acquiring more scans is the most straightforward, if rather time-consuming means of improving S/N (S/N increases as the square root to the number of scans. i.e. S/N ~$\sqrt{nt}$). An alternative approach is to apply a weighting function to the FID to improve Signal-to-Noise. Also, you can apply weighting functions to improve resolution, but with a concomitant loss of S/N. Take a

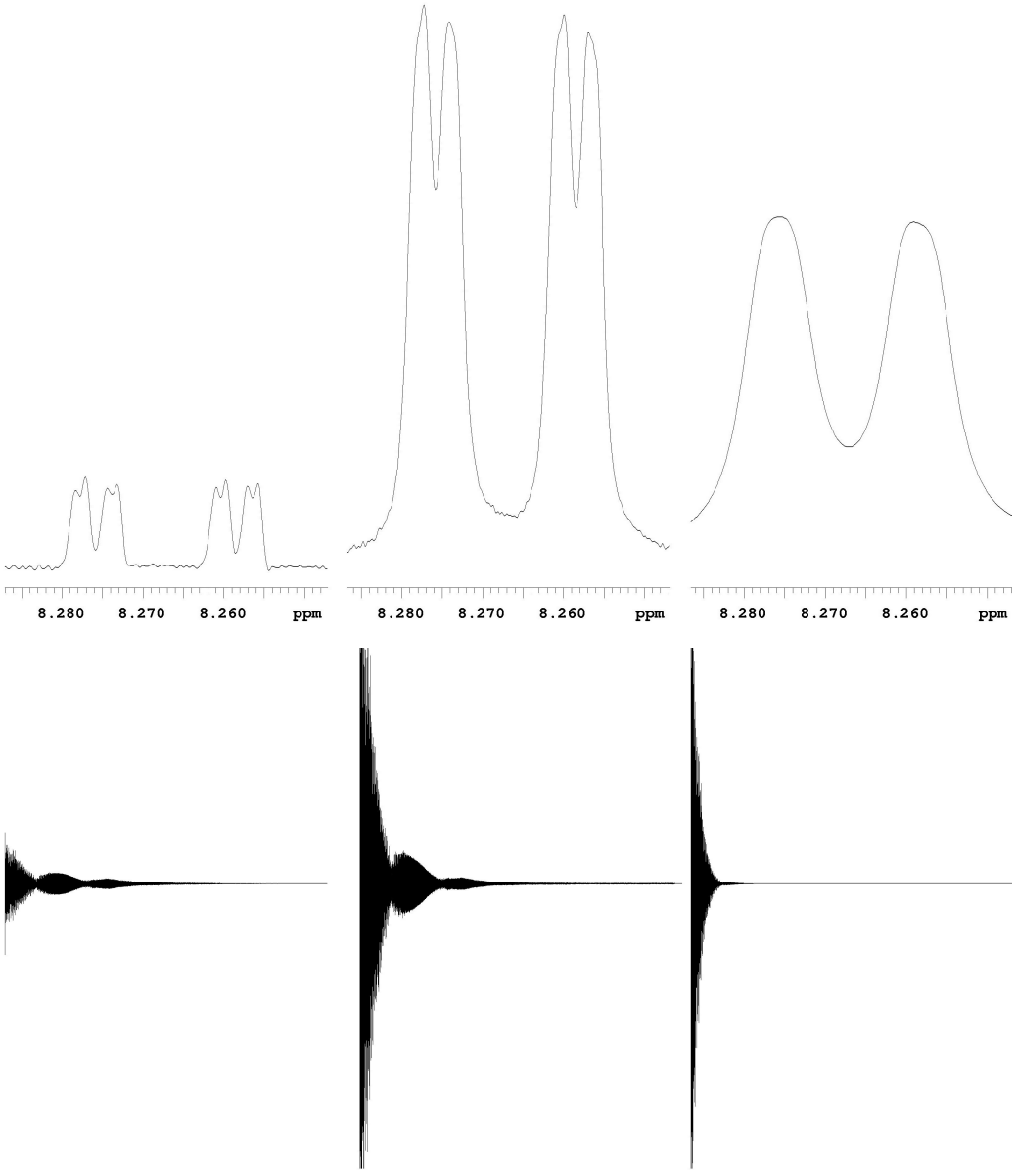


Figure 10. The effect of different weighting functions on an aromatic multiplet. Intensities are absolute. Note the differences in the FID's.

look at Figure 10. The three sets of peaks and their corresponding FID's are from the same experiment. The only difference between the peaks is the particular type of weighting function or apodization that was used. The set in the middle had no apodization and we see an apparent doublet-of-doublets (J = 8.7 and 1.7 Hz). The S/N for these peaks is 195.2 (the next section will describe the measurement of S/N). Since S/N is proportional to the initial intensity of the FID, multiplying the FID by an exponential curve [W(t) = exp(-lb$t$)], where lb is the line broadening factor, should result in improved S/N. Indeed, multiplication of the middle FID by the function with lb =2 gives the FID and spectrum on the right (see companion figure 11 as well). The S/N for these peaks is 878.13. It is more than a four-fold improvement in the S/N, but at the expense of line width. We have lost the small coupling constant, which is vitally

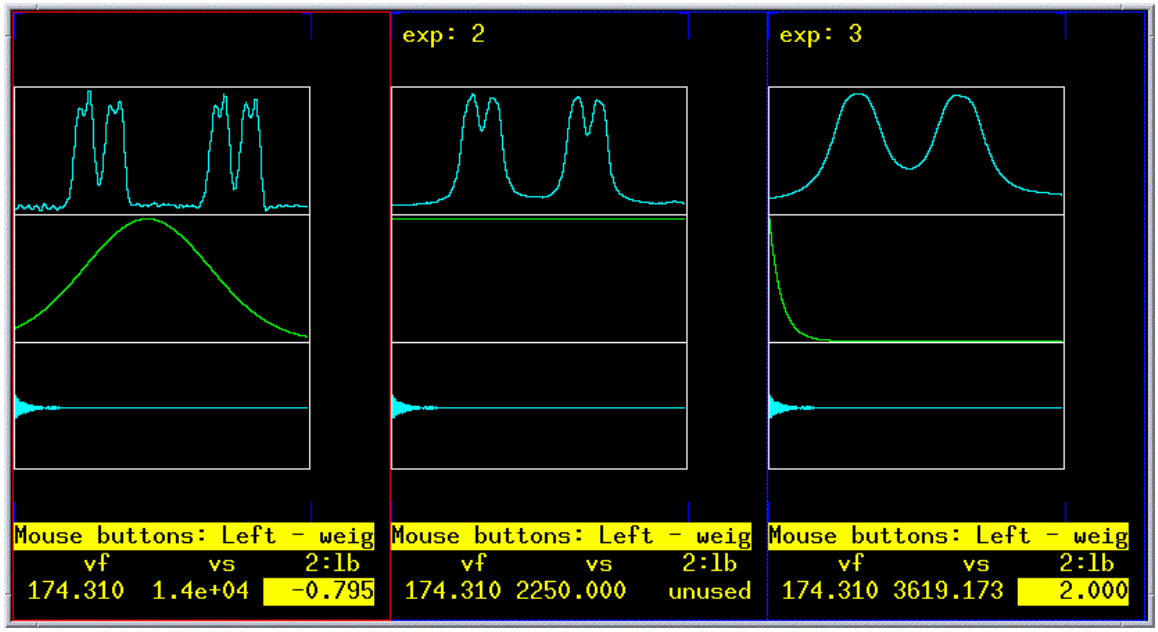


Figure 11. Interactive VNMR display of various apodization schemes. At top are the resulting spectra; middle is the weighting function; bottom is the raw FID's. Left: negative line broadening with Gaussian multiplication. Middle: no apodization. Right: 2 Hz line broadening.

important for structural elucidation. Exponential multiplication imposes an artificial rapid decay of the FID (compare the middle FID to that to the right). Since line width is inversely proportional to the transverse decay (T_2), a shorter FID means broader lines. In fact, exponential multiplication of this sort is termed line broadening, where lb will be the additional line width imposed by the function. Optimal S/N improvement occurs when

the lb factor equals the resonances' natural line width. Each resonance has its own line width and, therefore, a single lb value will not be optimal for every peak.

Apodization can also be used to improve resolution by emphasizing the tail of the FID. This has been done to the FID on the left of Figure 10. A function with a negative line broadening factor as well as a Gaussian function has been used (see Figure 11, for the VNMR interactive weighting window, which displays the function to the left). This has emphasized the middle and end of the FID and has revealed an additional coupling of 0.6 Hz. In effect it has extended the length of the signal. The price to pay for this apodization is a significant decrease in S/N; namely, from 195.2 to 60.8. Thus, you must use such weighting schemes with caution. Furthermore, apodization cannot make up for poor shimming or inadequate acquisition time. If it is not resolved in the time domain, it will not be resolved using either zero-filling or apodization.

Signal-to-Noise Measurement

The signal-to-noise measurement, or S/N, is an important criterion for accurate integrations, and is also one of the best ways to determine the sensitivity of a NMR spectrometer. In general, a higher S/N specification means that the instrument is more sensitive. It is also useful in roughly determining the time requirement for an experiment.

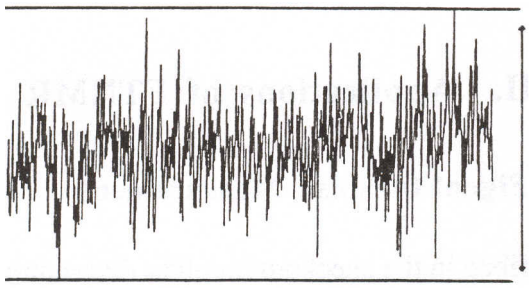
The standard manufacturer's S/N measurements for proton spectra are always determined using a sample of 0.1 % ethylbenzene in $CDCl_3$ (ETB). A typical result for the Varian Inova 500 is 250:1 using the 5mm Switchable BB probe.

Optimum signal-to-noise for any sample is achieved using a line broadening equal to the peak width at half height. When this line broadening is applied, the peak width at half-height doubles, i.e., it is the sum of the natural peak width at one-half height plus the line broadening applied. The equation used for calculating S/N is:

$$S/N = \frac{2.5A}{N_{pp}} \qquad (5)$$

(where A = height of the chosen peak and N_{pp} = peak-to-peak noise).

Peak-to-peak noise means exactly that - a measurement from the most positive to the most negative positions for the noise. As shown below, the widest differences are used for the measurement.



The distance between the two horizontal lines, above, in mm, is the N_{pp} value to be used in equation (5). Choice of a noise region must be consistently applied for standard samples. S/N measurement is an automated process and only requires choice of the appropriate window, placement of the cursors, and typing the correct command ('dsn' on Varians).

The signal-to-noise of a given signal increases as the square root of the number of acquisitions; therefore, to double the signal-to-noise you must take four-times as many acquisitions. When using a concentrated sample such as 57% menthol for ^{13}C, or when running routine ^{1}H spectra, the number of scans is often quite small, so the point discussed above may not seem important. However, suppose you are in the following situation: you have only a few mg of research sample, and after collecting a ^{13}C spectrum for 2 hours, you get peaks with an S/N of only 5:1. Since the peaks are barely visible above the noise (and you may have missed any quaternary carbons), you want to re-collect the spectrum to get an S/N of 50:1, a value more typical for carbon NMR. Unfortunately, this will take 10 * l0 * 2 = 200 hours! At this point alternatives would be considered, including more sample, specialty tubes like a Shigemi NMR tube, higher field spectrometer, use of relaxation agents, and/or a cryo-probe.

S/N Take Home Lesson

At some point, you may take a spectrum and wonder why the signals are so weak.

Over 85% of the time, the problem is not with the spectrometer, but with your sample. You can test this quickly by taking a spectrum of a standard such as ETB or menthol. In this way, you can save yourself needless frustration by identifying problems that are due to a bad sample. Always obtain the spectrum of a standard, well-characterized compound before obtaining that of your unknown.

Integration

The purpose of this section of the handout is to show you how to obtain accurate integrals. With a properly setup ^{1}H NMR experiment; the integrated area under the resonances is proportional to the number of nuclei responsible for that peak. Improper settings lead to incorrect results. For example, the spectrum of 0.1 % ethylbenzene in CD_2C1_2 is given in Figure 12. $CDCl_3$ is not used in this case because the solvent peak overlaps with the phenyl region and obscures integration. If we assign an integral of 3.00 to the CH_3 triplet; the phenyl region integrates to 4.12 protons, while the CH_2 quartet integrates to 1.93 protons. Thus, the integral for the phenyl protons is 17.6% too small, while the integral for the CH_2 quartet is off by only 3.5%. The 17.6% error for the phenyl protons is not due to spectrometer error, it is because we have chosen parameters for acquiring the spectrum that guarantee we will get inaccurate integrals. If this were an unknown sample, you would think that the aromatic ring had double substitution and would, needlessly, be trying to derive an incorrect structure.

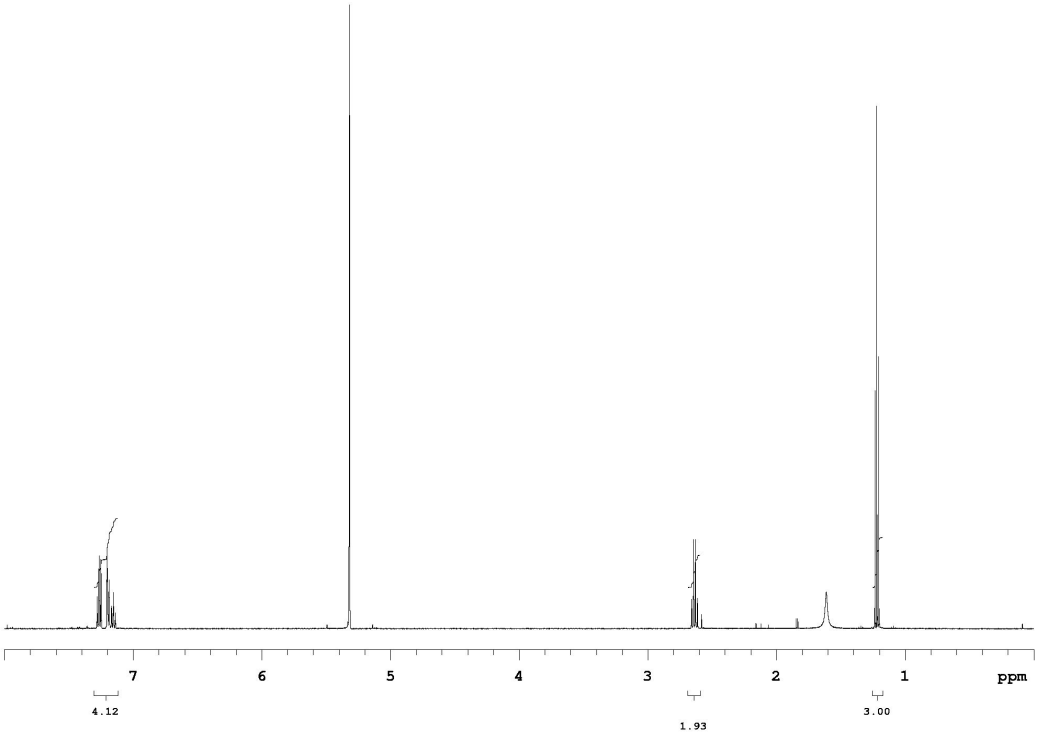


Figure 12. ^{1}H NMR spectrum of 0.1% ethylbenzene in CD_2Cl_2 taken on a Varian Inova 500 MHz spectrometer with no recycle delay (nt=4, d1=0). The longest T_1 for this sample was measured at 12 seconds.

The accuracy of the integrals obtained for most routine spectra is usually about 80-90%. This accuracy is sometimes sufficient, especially if you already know the compound's structure. However, this accuracy is usually not adequate to determine the exact number of protons contributing to a given peak, nor is it sufficient for quantitative applications (such as kinetics experiments or assays of product mixtures) where one demands an error of 1-2%. For example, 80% accuracy is not sufficient to decide whether two peaks have a relative ratio of 1:3 or 1:4. Obtaining 1-2% error can be achieved but you need to be aware of the factors that affect integrations. These are as follows:

I. There should be no nuclear Overhauser effect contributions or any other effects that selectively enhance certain peaks. This is a problem only with X nuclei such as ^{13}C and will be dealt with in section 4.

II. No peaks should be close to the edges of the spectrum. The spectral width should be large enough such that no peak is within 10% of the ends of the spectrum. This is because the spectrometer uses filters to filter out frequencies that are outside the spectral width. Unfortunately, the filters also tend to decrease the intensities of peaks near the ends of the spectrum. For example, at 500 MHz, if two peaks are separated by 7 ppm, a spectral width of at least 3500 Hz is sufficient to get both peaks in the same spectrum and prevent foldovers. However, to avoid distortion of the integral intensities because of filter effects, the spectral width should be set 10% larger on each side, 350 Hz, giving a total spectral width of about 4200 Hz (8.4ppm). Thus, you should be prepared to make the spectral width larger if necessary.

III. The recycle time should be at least five longest T_1's. Data should be collected under conditions which ensure that all the nuclei can fully relax before the next FID is taken, i.e., if 90º pulse widths are used, relaxation delays of FIVE times the longest T_1 of interest are necessary. In the case of the 0.1% ethylbenzene in CD_2C1_2 in Figure 12, the longest T_1 of interest is 12 sec (phenyl protons), so the relaxation delay when using a 90º pulse width should be 60 seconds.

IV. The spectrum should have a S/N of at least 250:1 for the smallest peak to be

integrated. Typically, if you cannot see any baseline noise, you probably have close to the required S/N for accurate integrals.

V. The baseline should be flat. Distortion due to phase problems should be corrected. Baseline distortion due to non-optimum parameter selection that causes a baseline roll will not be discussed here. See lab staff for help if you suspect this problem.

VI. The peaks need to be sufficiently digitized, as discussed earlier in this handout. If the linewidth at half-height is 1 Hz, you need a digital resolution of less than 0.5 Hz.

VII. The same area should be included or excluded for all peaks. For example, all peak integrals should be measured +/- 5 Hz around each peak, not +/- 20 Hz around one peak, +/- 10 Hz around a second peak, etc. Spinning sidebands are included in this category, and should consistently be either included or excluded.

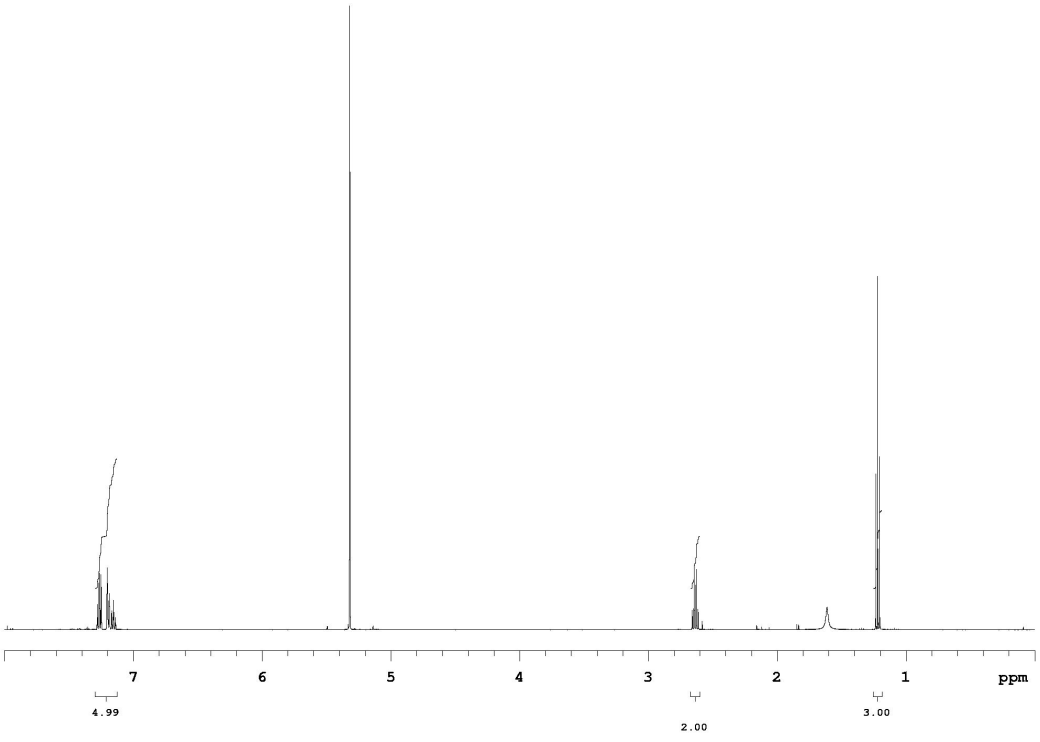


Figure 13. ^{1}H NMR spectrum of 0.1% ethylbenzene in CD_2Cl_2 taken on a Varian 500 MHz spectrometer with a recycle delay of 60 seconds (nt=4, d1=60). The longest T_1 for this sample was measured at 12 s.

With these points in mind, let's take the ^{1}H spectrum of ethylbenzene again. The major factor for poor integration in Figure 12 was the difference in T_1's for the aromatic protons (~12 seconds) and the aliphatic protons (~7 seconds). With no recycle delay, there was not enough time to allow for complete relaxation. If we allow for complete relaxation by setting d1 large enough, say 60 seconds, then integration becomes accurate as shown in Figure 13 with only a 0.2% error of the aromatic protons.

Sometimes, we do not want or need perfect integration and can accept some error in the results. You can calculate the necessary repetition rate (i.e. the recycle delay plus acquisition time or TT) to give acceptable quality. The formula to calculate the repetition rate is:

$$TT = \left(\frac{1 - Q}{1 - Q \cdot cos(\alpha)} \right) \cdot T1$$

Q is the quality (e.g. 0.95 for 95%); *T1* is your T_1 time (or estimated longest T_1), and α is the pulse angle (typically 45º).

Integration Take Home Lesson

Taken from Derome (p. 172)

"The moral of this section is that there are numerous contributions to the error in a quantitative measurement made by FT NMR, and while each of them may be reduced to 1% or so in a practical fashion, the combined error is still likely to be significant. I am always skeptical of measurements purporting to be accurate to better than a few percent overall, unless they come with evidence that careful attention has been paid to the above details."

Proton Decoupled ^{13}C NMR spectra ($^{13}C\{^{1}H\}$)

The purpose of this section of the handout is to give you some useful information about $^{13}C\{^{1}H\}$ NMR spectroscopy. Since only about 1 in 100 carbon nuclei are NMR active (1.10% are the NMR active ^{13}C isotope), any means to improve S/N is essential. Splitting of the ^{13}C resonances as a result of coupling to attached protons will result in decreased S/N and is, thus, undesirable. Therefore, ^{13}C NMR spectra are typically run proton decoupled. The symbol $^{13}C\{^{1}H\}$ is used to denote this and implies the ^{13}C nucleus is observed while the proton nuclei are being irradiated and decoupling them from the ^{13}C nuclei. A typical $^{13}C\{^{1}H\}$ spectrum (57% menthol in acetone-d_6) is shown in Figure 15.

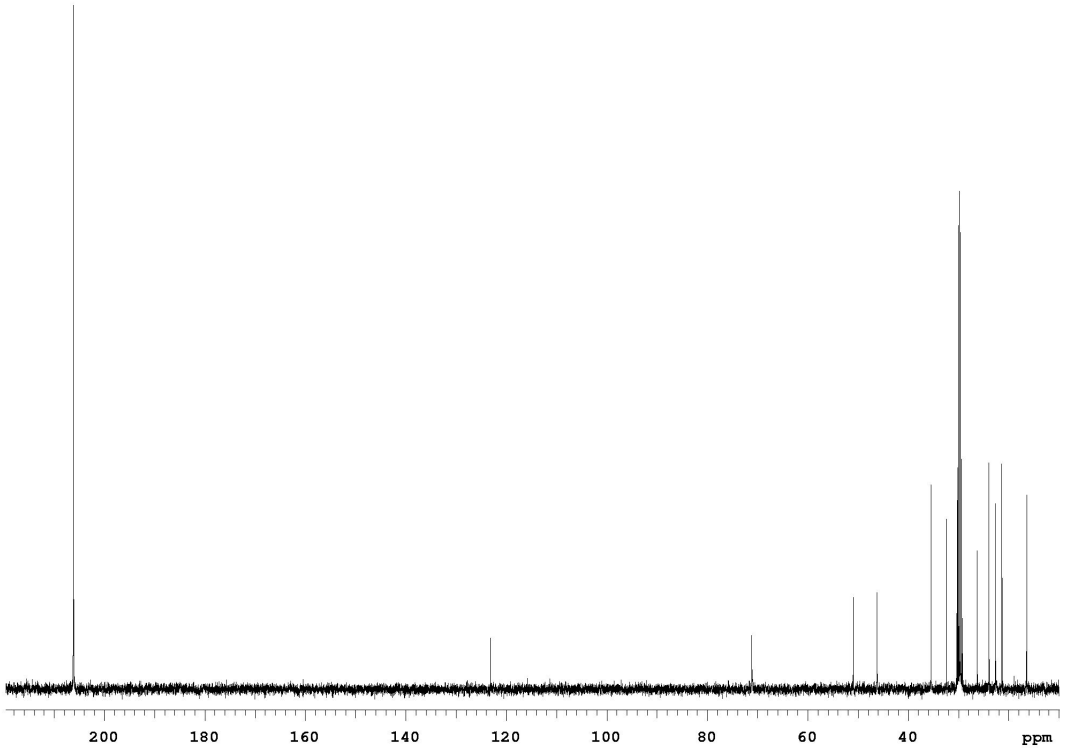


Figure 15. A $^{13}C\{^{1}H\}$ NMR spectrum of a 57% solution of menthol in acetone-d_6 acquired on a Varian Unity 400 MHz spectrometer.

This is a double resonance experiment with the observed nucleus (^{13}C) and decoupled nucleus (^{1}H) on separate channels. This experiment is called heteronuclear

decoupling, and is a '1PULSE' experiment, as described in the Basics section, with the addition of a decoupling field, as shown in Figure 16.

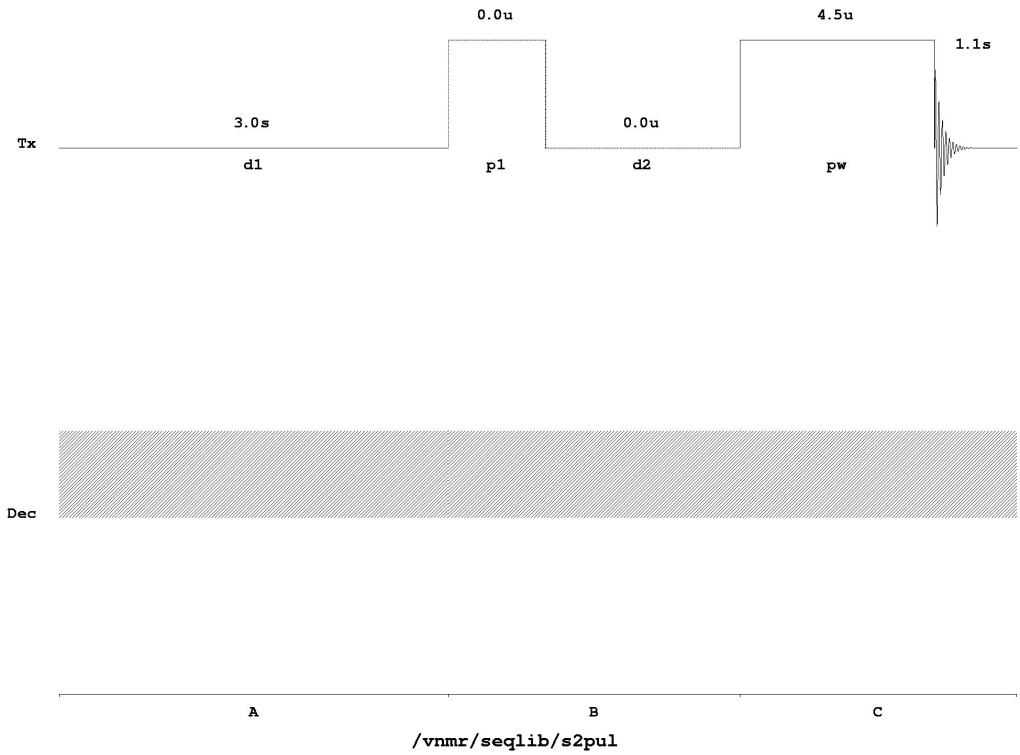


Figure 16. Representation of $^{13}C\{^{1}H\}$1PULSE NMR experiment as presented on a Varian Inova 500 MHz Spectrometer. Note that the p1 pulse has a pulse time of 0.0 and is unused in this sequence.

When acquiring spectra of nuclei other than protons (so called 'X- nuclei') it is important to remember the following considerations:

I. **The Nuclear Overhauser Enhancement**: The $^{13}C\{^{1}H\}$ NMR spectrum obtained using a standard 1PULSE experiment is <u>not</u> quantitative, i.e., the integration of the peaks will not give a true indication of relative ratios because, among several factors, of a phenomenon called nuclear Overhauser enhancement (NOE) arising from the continuous broadband saturation of the protons. ^{13}C nuclei that have directly bonded protons can exhibit a signal enhancement of up to 1.98 (198%), or an almost threefold improvement in

signal-to-noise. The NOE is from the dipolar through-space coupling of the carbon and proton nuclei and is dependent on many factors. Thus, the NOE will be different for each unique carbon in a molecule. To obtain quantitative $^{13}C\{^{1}H\}$ spectra, you must do two things: follow the protocol given earlier on integration, and carry out a 'gated' decoupling experiment, in which the decoupler is gated on (turned on) during the acquisition time and gated off (turned off) during the recycle delay. This is shown in Figure 17.

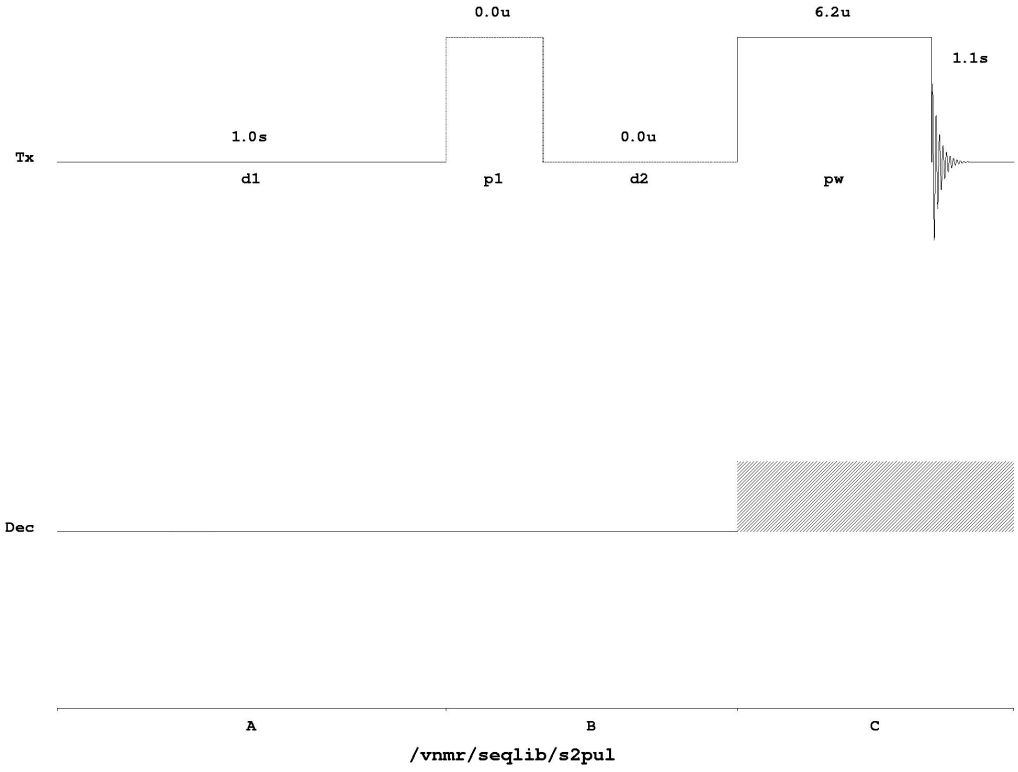


Figure 17. A gated decoupling pulse sequence for $^{13}C\{^{1}H\}$ acquisition that has no nOe enhancement. Note that the decoupler channel (Dec) is only 'on' during segment C, which is the pulse and acquisition time. Compare to Figure 16.

The result of this experiment is a $^{13}C\{^{1}H\}$ spectrum without NOE and is necessary for obtaining quantitative ^{13}C spectra.

II. **T_1 relaxation times:** The T_1's of ^{13}C nuclei are in general longer than those

found for protons, as shown below in Figure 18. Therefore, you may have to wait very long times if you want accurate integrals from spectra. For example, from Figure 18, quantitative integration of ethylbenzene would require a total acquisition time (TT) of 5*36 seconds or 3 minutes per scan! A paramagnetic relaxation agent such as Cr(acac) (available from Aldrich) can be used to shorten the T_1's, but can sometimes be difficult to separate from the compound. Note that the quaternary carbons have considerably longer T_1's and, as a result, typically have much smaller signals than other carbons.

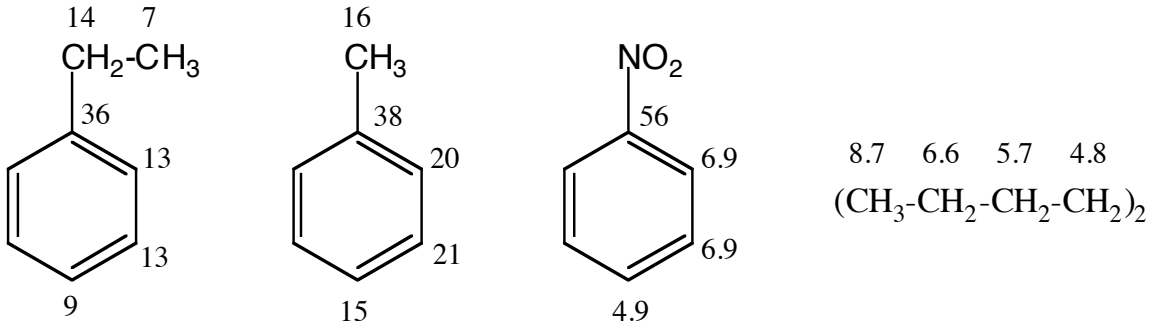


Figure 18. Examples of some representative ^{13}C NMR T_1 values, in seconds.

^{13}C NMR Take Home Lesson

Obtaining useful $^{13}C\{^1H\}$ spectra requires knowledge of the same basics as needed for obtaining useful ^1H spectra. When your spectrum doesn't look right, you can save frustration on the instrument be taking a quick spectrum of a ^{13}C standard and checking the S/N, or seeing if the standard is decoupled properly.

$^{13}C\{^{1}H\}$ DEPT Spectra

Distortionless **E**nhancement by **P**olarization **T**ransfer (DEPT) is an experiment that utilizes a polarization transfer from one nucleus to another, usually proton to carbon or other X nucleus, to increase the signal strength of the X nucleus. DEPT is an example of a multi-pulse, multi-channel experiment, which uses synchronous pulses on two channels to afford polarization transfer. The pulse sequence is shown in Figure 19. In this case, the decoupler channel (Dec) is proton and the observe channel is carbon. Since we are transferring the population difference of the protons to the X nucleus and gaining signal from these protons, it is the proton T_1's that are important in determining repetition

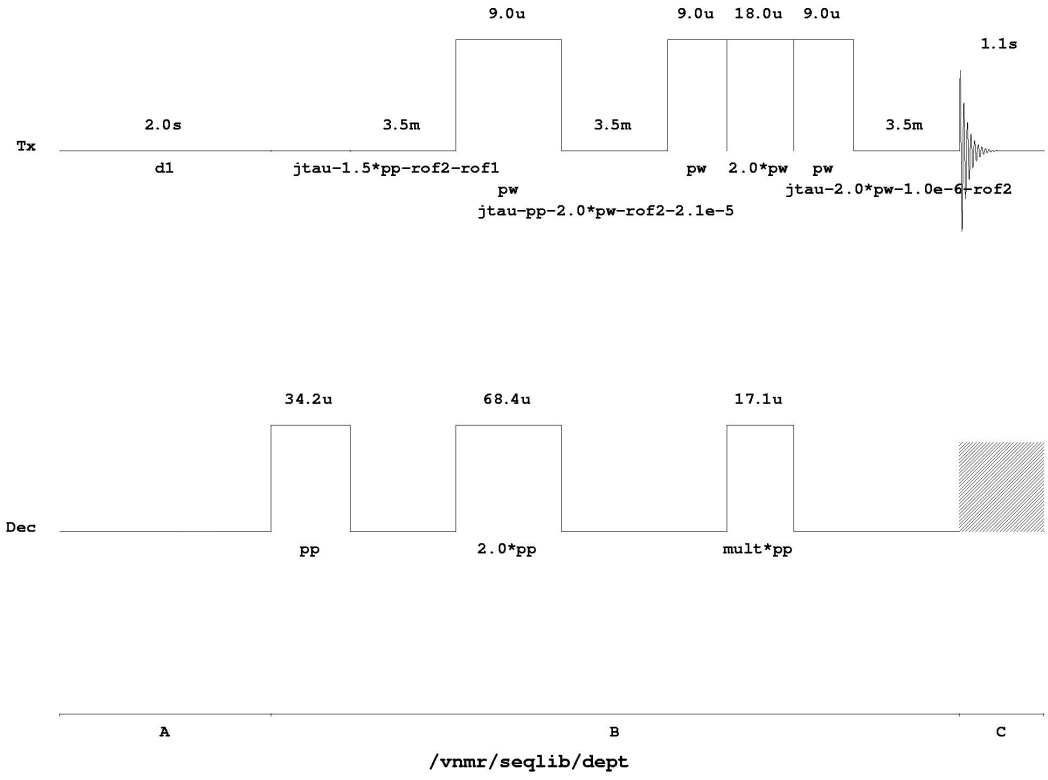


Figure 19. Schematic representation of a DEPT pulse sequence as displayed by a Varian Inova 500 MHz spectrometer. The observe (Tx) channel is carbon and the decoupler (Dec) channel is proton.

rate for a DEPT experiment. Proton T_1's can be significantly shorter than that of carbon and especially short compared to ^{15}N and ^{29}Si, which allows you to acquire more scans

per unit time than the X{^{1}H} experiment and thus obtain improved S/N. A further advantage of this population transfer is the ability to perform multiplicity editing.

By varying the length of the last proton pulse (mult*pp) from 45 to 135º degrees, the multiplicity of the carbon or X nucleus can be determined (i.e. depending on the pulse, the signal for a methine, methylene, or methyl will either be a positive, negative, or null signal, see table below). Remember, since quaternary carbons have no attached protons, they will show no signal. Also, the signal from the deuterated solvent will be absent. An example of a DEPT135 experiment is shown in Figure 20. Compare it to Figure 15. In general, you can run DEPT on most samples without additional calibration.

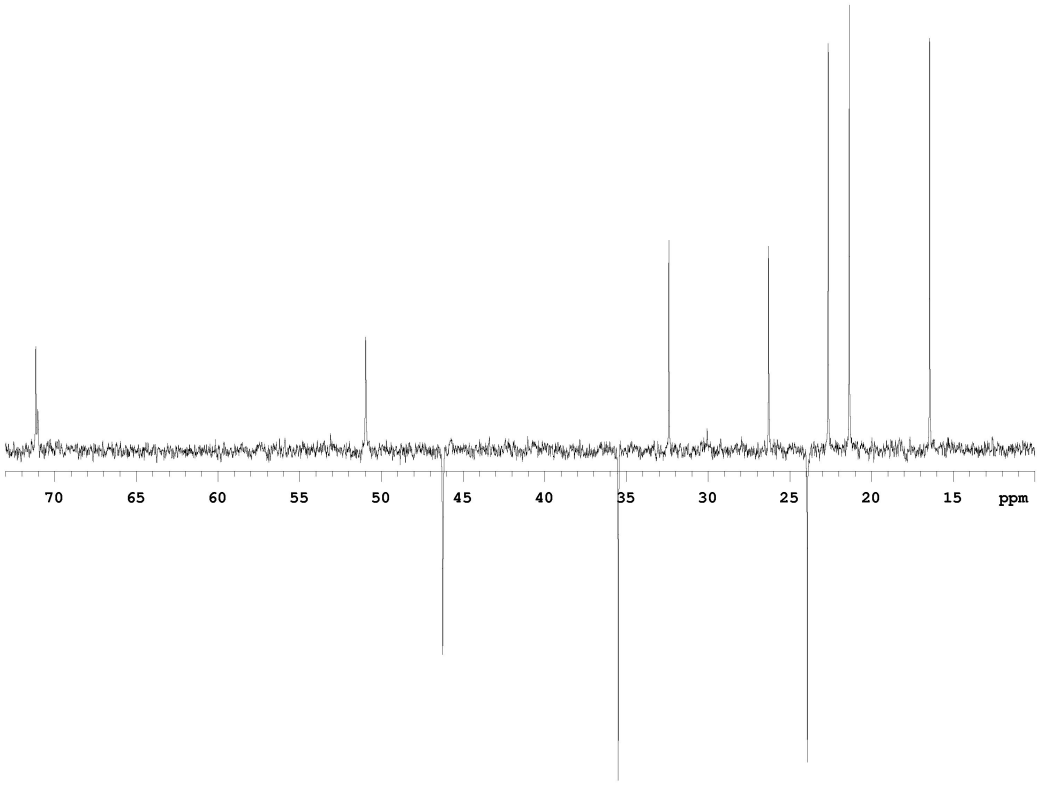


Figure 20. An example of a multiplicity-edited spectrum. Expansion of a ^{13}C-{^{1}H} DEPT-135 spectrum of menthol taken on a Varian Unity 400 MHz spectrometer. The relative sign of the peaks shows the multiplicity of the carbons. The negative peaks are methylene carbons, while the positive peaks are either methyl or methine carbons.

If you obtain less than favorable results, calibration of the polarization pulse (pp on the Decoupler channel) can be performed. This is typically done using a DEPT-90, arraying

pp, and looking for a maximum in the methine signal without contributions from other carbons.

Relative Intensities from DEPT				
Pulse Angle (º)	C (quaternary)	CH (methine)	CH_2 (methylene)	CH_3 (methyl)
45	0	0.707	1	1.06
90	0	1	0	0
135	0	0.707	-1	1.06

DEPT Take Home Lesson

DEPT is an effective means of determining ^{13}C multiplicity that, when combined with other NMR spectra and other experimental techniques (MS, FT-IR, etc.), can be an invaluable tool for the analysis of unknown compounds.

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NMR Basics Test

1. Diagram and label the three parts of the 1PULSE FT NMR experiment.

2. What is the result when you apply FT to a FID (time domain signal)?

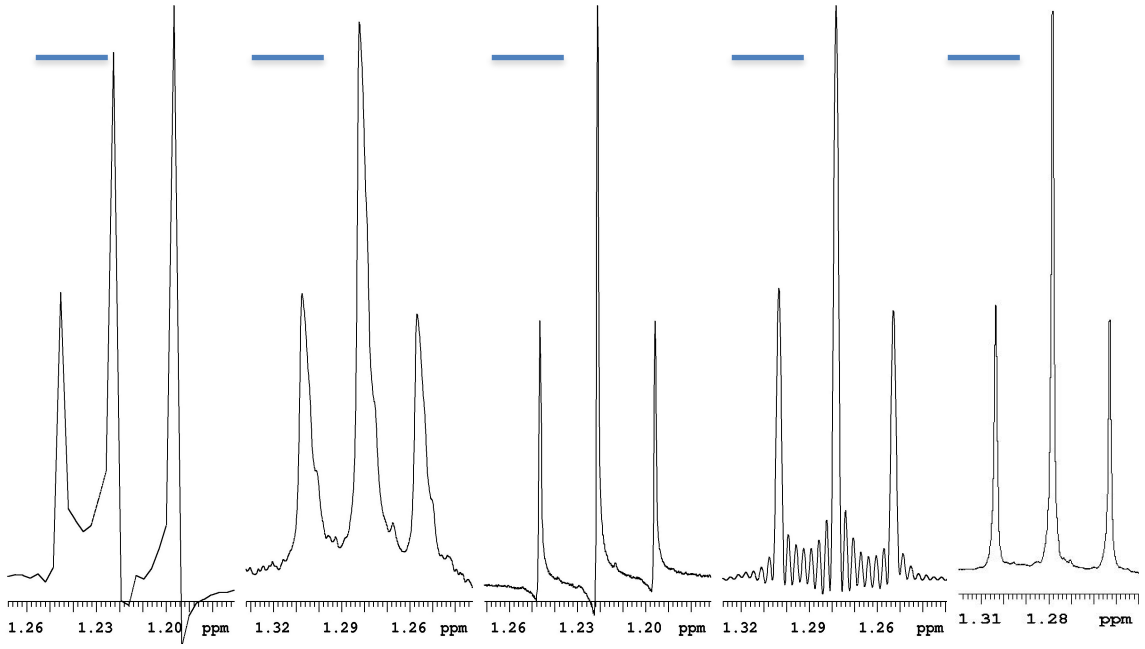
3. What is the relationship between numbers of points, spectral width, acquisition time, and digital resolution? What is the rule-of-thumb for adequate digital resolution? Which of these parameters would you change if you wanted better digital resolution, and why?

4. What is the peak shape found in most solution NMR spectra?

5. What shim(s) should be adjusted if the peak shape is asymmetrically distorted?

6. What is the single best factor to tell whether a sample is poorly shimmed?

7. Label the spectral artifact with its cause (use the upper case letter corresponding to the cause of the artifact).



A. Improper Phasing B. Good Spectrum C. Low Resolution D. Poor Shimming E. FID Truncation

8. What type of apodization would you use to improve signal-to-noise (S/N)? What is the disadvantage?

9. Given that after 100 scans (5 minutes) the S/N for a sample is 35: 1, how long will it take to achieve a S/N of 350:1?

10. What are six factors that can affect the accuracy of a ^{1}H NMR spectral integration? Why? Are there any additional factors that affect the accuracy of ^{13}C- $\{^{1}H\}$ integration? Why?

11. Is there a difference between the 1PULSE FT NMR experiment used to acquire ^{13}C-$\{^{1}H\}$ NMR spectra and that used to acquire proton spectra? If yes, what is the difference?

12. For a DEPT135 spectrum, what are the relative intensities for the different multiplicities of carbon?