Basic 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. 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.

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Introduction

Nuclear Magnetic Resonance (NMR) is a powerful relatively non-selective analytical tool, which enables you to ascertain molecular structure including relative configuration, relative and absolute concentrations, and even intermolecular interactions. Once challenging and specialized NMR techniques have become routine. NMR is indeed an indispensable tool for the modern scientist. Chemists, with little knowledge of NMR, are now able to obtain 2- or even 3-dimensional spectra with a few clicks of a button. 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, NMR system, or hardware configuration, they may be very wrong for another. A single incorrectly set parameter can mean the difference between getting an accurate, realistic spectrum and getting a meaningless result, or even worse, damaging equipment that often costs several hundreds of thousands of dollars. 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. It is not meant to replace any formal training of the theory of NMR.

"Modern pulse NMR is performed exclusively in the Fourier Transform 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 Width [**sw**]

6. Recycle Delay [**d1**]

[The **blue** letters in square brackets following the parameter represent the mnemonic used (*the command*), on all Varian spectrometers in the VNMR software package. The parameters are discussed in more detail below.]

The most basic and common pulse sequence you will encounter is the '1-PULSE' FT-NMR experiment (e.g. **seqfil = 's2pul'** in VNMR), which is the sequence used for routine ^{1}H and ^{13}C 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 the signal-to-noise ratio (S/N), which increases with the square root of the number of transients that are averaged together (**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 spectrometers, you can view the current pulse sequence by typing '**dps**'.

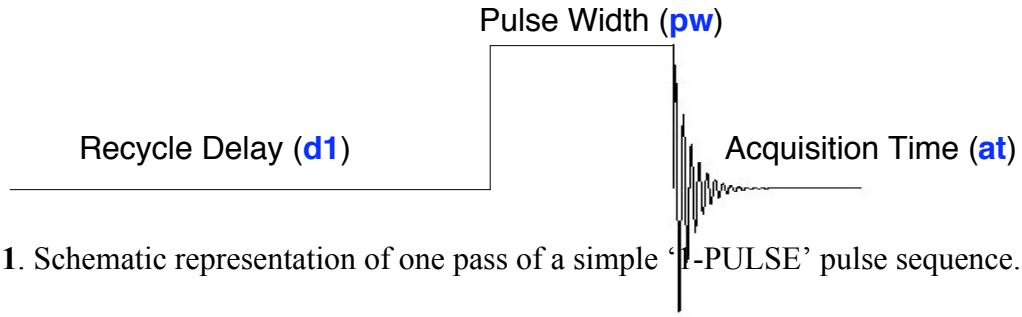


Figure 1. Schematic representation of one pass of a simple '1-PULSE' pulse sequence.

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

It is called a "1-PULSE" experiment because one radio frequency (Rf) pulse (**pw**) is applied per cycle. The Rf pulse excites the nuclei, which then emit Rf during the acquisition time, giving rise to an NMR signal in the form of an exponentially decaying sine wave, termed free-induction decay (FID). The Rf 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 hydrogen atoms resonate. Thus, a Varian INOVA 500 will cause hydrogen atoms to resonate at approximately 500 MHz. The precise spectrometer frequency defines the exact center of the NMR spectrum you measure.

An Rf pulse that excites spins at only one exact frequency is not desirable because the NMR frequencies are spread out over a range of frequencies called the range of chemical shifts (corresponding to ~10 ppm of the applied magnetic field for ^{1}H and ~250

ppm for ^{13}C). Luckily, the short pulse lengths used in FT-NMR (e.g. ~10 μs) causes the Rf power to be distributed over a corresponding frequency spread (~1/**pw**, or 1/0.00001 seconds = ~100,000 Hz) due to the Heisenberg Uncertainty Principle. As you shorten the pulse length and increase power, uncertainty in the frequency results in a larger field of excitation (frequency range of power deposition). A longer, lower power pulse will have less frequency spread and can be used for frequency selective excitation or saturation.

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

Prior to applying an Rf pulse, the majority of nuclear spins are aligned parallel to the static magnetic field (B_0). 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 Rf pulse at the appropriate frequency will rotate the bulk magnetization by a specific angle. Pulses are generally described by this angle of rotation (also called flip angle). The angle of rotation is dependent on the intensity of the pulse (**tpwr**) and the width of the pulse (**pw**). The maximum measurable signal can be recorded following a 90º rotation or pulse. Thus, a 90º pulse width is defined as the amount of time the pulse of Rf energy is applied to the particular sample (90º is not 90º for all samples!) in order to flip the bulk magnetization from the Z-axis precisely into the X-Y plane, i.e., the condition shown in Figure 2A. For example, the 90º pulse width for ^1H NMR experiments might be about 8 μs on the Varian Inova 500. It is often very useful to talk about the strength of the oscillating magnetic field associated with the Rf pulse in terms of the rotation frequency (rather than the time required to rotate the bulk magentization 90º). For example, if it takes 8 μs to rotate 90º, then using the same Rf intensity it will take 4*8μs = 32 μs to rotate 360º. The intensity of the Rf field can then be described as a magnetic field that causes the magnetization to rotate at a rate of 1/32 μs, or at 31,250 Hz (31.25 KHz). We call this magnetic field the B_1 field, analogous to our description of the main static magnetic field (B_0). (Note: a 300, 400, or 500 MHz static B_0 field is roughly 10,000-20,000 times stronger than a typical oscillating B_1 field that comes from the Rf pulse). The importance of learning this convention will become strongly apparent when you setup a homonuclear decoupling or nOe experiments, or perhaps a two-dimensional TOCSY experiment.

The pulse width is entered in microseconds by typing **pw**=desired value in microseconds. 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, not required until you attempt advanced experiments. For routine one-dimensional survey experiments, most users use a 25 - 45º pulse for their data collection (Figure 2B). The reasons for this are discussed under recycle delay (**d1**).

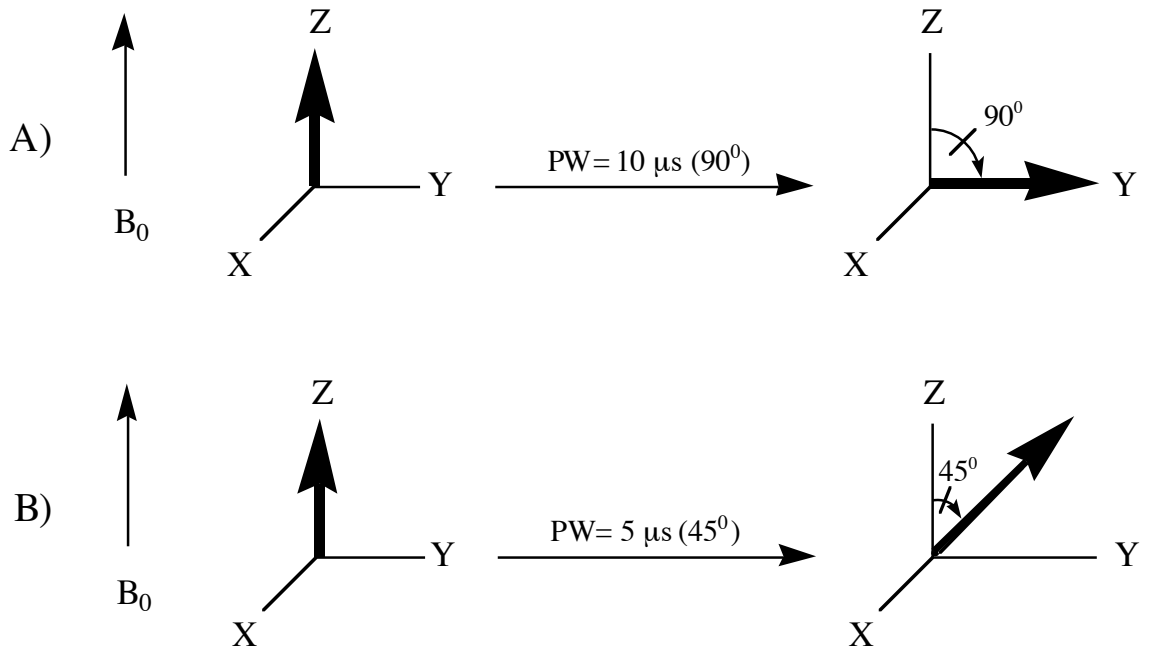


Figure 2. The bulk nuclear spin magnetization (***bold arrow***) for an NMR sample placed in a magnetic field aligned along the Z-axis before and after application of a pulse.

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

Thus far, we have sent an Rf pulse through the sample and flipped the bulk magnetization by a specific angle. The nuclear spins are no longer at equilibrium and will eventually return to equilibrium along the Z-axis. One of the most extraordinary aspects of NMR is that the relaxation back to equilibrium takes from 10^6-10^{12} times longer than many other spectroscopies that you routinely utilize! 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 it takes to acquire the FID is called the **acquisition time** and is set by the parameter '**at**'. For most nuclei of interest to chemists, relaxation times are within the range of 10^{-1} to 10 seconds. One of the most important concepts that chemists should learn is that routine matching of the acquisition time to the full decay time of the FID is a primary goal in recording quality NMR data. A natural inclination might be to just increase the acquisition time to maximize the amount of signal that is acquired (up to some "safe" limit). However, increasing the acquisition time is only advantageous up to a point. Moreover, it 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 show up at the base of the peaks.

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 into a series of discrete points along the FID signal envelope. 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**),

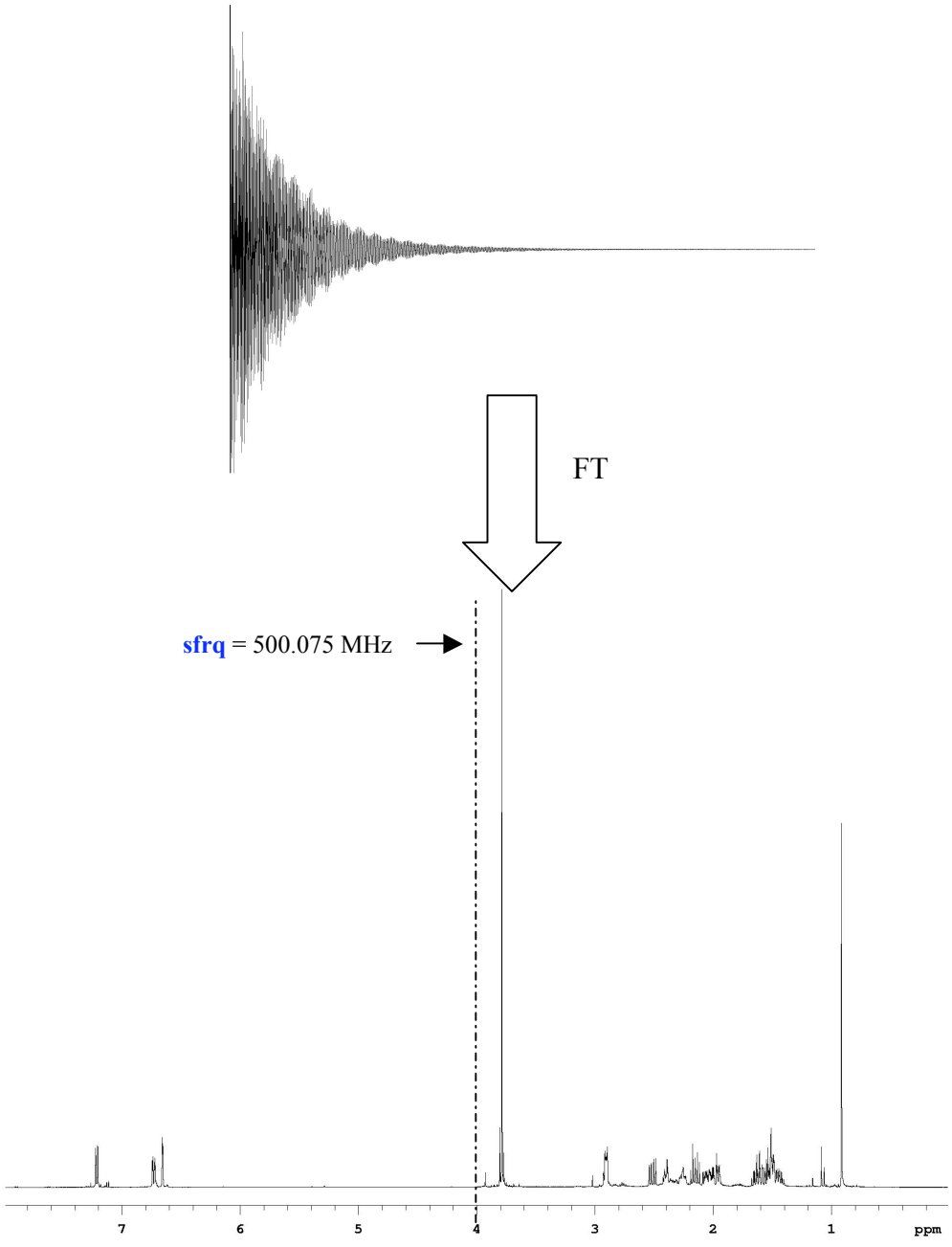


Figure 3. Fourier transform of the FID for estrone acquired at 500 MHz. Note: the spectrometer frequency you use, in general, will not be exactly 500 MHz.

spectral width (**sw**), and acquisition time (**at**) are interrelated. Changing one of these parameters will affect the other two (see below).

5. **Spectral 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 time-domain (seconds) FID (commonly referred to as FT or FFT for Fast Fourier Transform) will produce a frequency domain (seconds^{-1}) spectrum with the familiar peak shapes and intensities, 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 (*often times erroneously called the 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. For example, at a spectrometer frequency of 300 MHz, a spectral width of 3000 Hz is needed to measure a 10 ppm frequency range, 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 5000 Hz is needed to measure 10 ppm (10 ppm x 500 Hz/ppm).

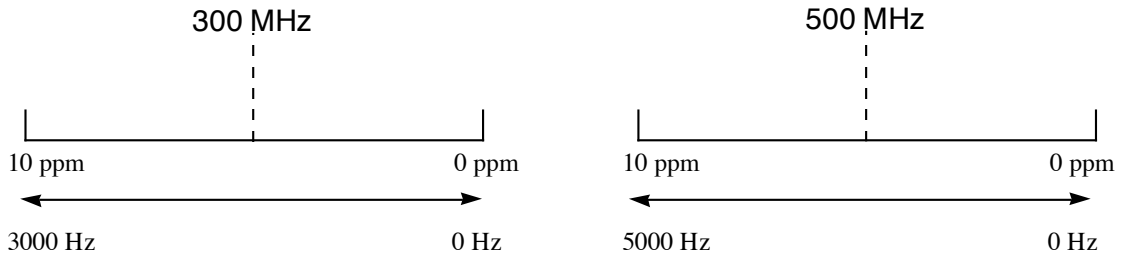


Figure 4. The spectral width in ppm and Hertz at different spectrometer frequencies. Note the difference in the spectral width in Hertz for the two spectrometers.

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

$$at = \frac{np}{2sw} \tag{1}$$

and

$$res = \frac{1}{at} = \frac{2sw}{np} \tag{2}$$

where '**res**' refers to 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 natural 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:

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

Since the computer works most efficiently if the number of points is 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, Varian's automatically change the number of points according to equation 2 if the acquisition time or sweep width are changed.

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

On Varian's this delay time is named **d1** 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 approach equilibrium; rather, they each relax according to a time constant called T_1 (longitudinal relaxation time, which parameterizes any approximation of the time necessary for the magnetization to approach the Z-axis). Individual T_1 time constants are dependent on many factors including nuclear environment, temperature, and solvent. T_1's for carbon atoms are typically much longer than T_1's for hydrogen atoms. 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). When a 90° pulse is used to excite the spins (Figure 2A), a total time (TT) between pulses of $5xT_1$ is necessary in order to have nearly complete relaxation. (Z-magnetization = $(1 - e^{-(\text{elapsed time}/T1)})$, where $1 - e^{-5} = \sim .99326$). If a pulse width less than 90° is used, the total time can be proportionally less, and intensity distortions due to differential relaxation effects can be minimized. This is one reason why the standard pulse width for 1D ^{1}H NMR experiments are in the range of 25 - 45º.

The total time between measured transients 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 usually be ignored, leaving us with the equation:

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

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

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

As an example of the above, if your longest T_1 is 600 msec, then the total time (where

TT=5x T_1) must be at least 3 seconds, to completely avoid relaxation based distortions of signal intensity.

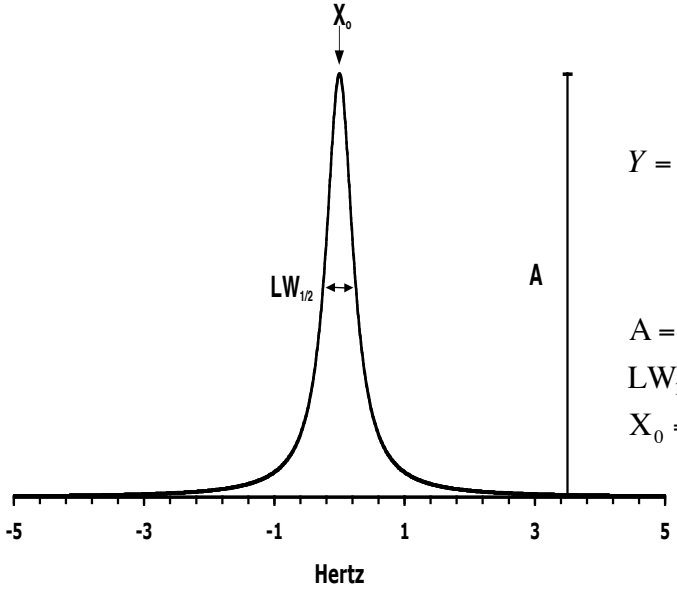
Take Home Lesson

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 1-PULSE experiments, but also is equally important in 2-D and 3-D NMR spectroscopy.

Applications of FT-NMR

1. $CHCl_3$ 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.



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

A = Amplitude

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

X_0 = Peak position, in Hz

Figure 5. 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, although peak widths of less than 0.10 Hz are obtainable by an expert shimmer. 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 (see 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 line shape 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.

Shimming:

The term 'shimming a magnet' is a piece of NMR jargon that harks 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.

It is important for you to have a basic understanding of line shape 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 file that matches the current probe (e.g. **probe = 'as'**) when you sit down at the instrument. You should never assume the previous user left the instrument with a standard shim file 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 (*as most novice users will*). 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.

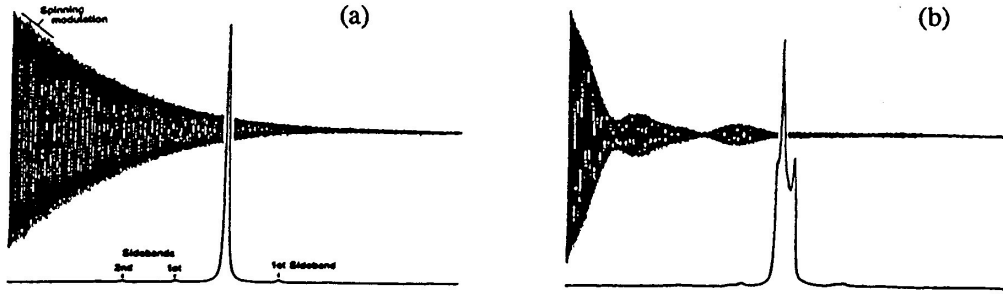
III. Shimming problems are confirmed only if the problem is visible on <u>every</u> peak in your spectrum. If, for example, only one peak is doubled, the problem is sample related, and can't be shimmed away. Remember, anomalies close to the base of intense single lines may not be visible on less intense peaks unless the vertical scale is increased.

IV. Establish a shimming method. Shimming is an 'art form' that requires

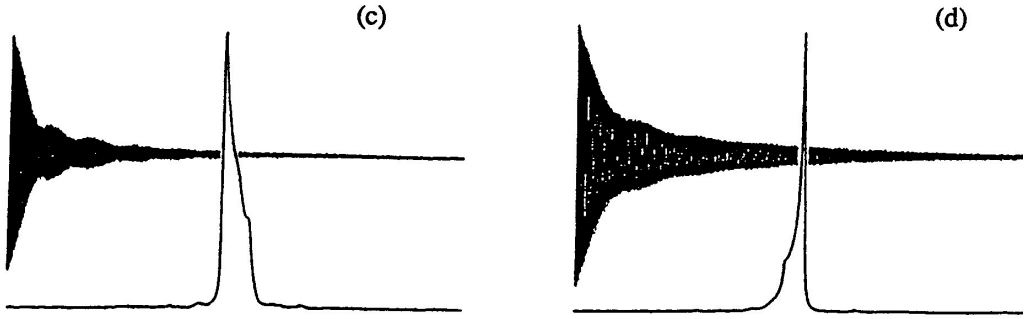
patience and practice. You should always approach shimming with some method that works for you to give acceptable results. Example: load a shim file; adjust the lock level to a maximum with Z_1, then Z_2, then Z_1, then Z_3, and then Z_1.

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 Z_1, Z_2, and Z_3. If this does not solve the problem, consider transferring your sample to another tube.

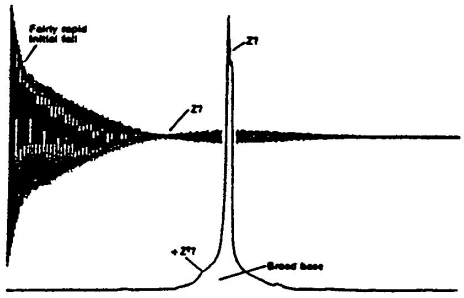
Knowledge of correct line shape can help you correct problems such as those shown in Figure 6. Although the peak in Figure 6b may have a line width at half-height that is less than 0.50 Hz, it is obviously poorly shimmed. You should never accept a poorly shimmed line shape such as is shown in Figure 6b, where a single line is expected. On the pages that follow are some line shape 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 longitudinal shims (Z_l, Z_3, Z_5) affect the line shape symmetrically while even-order longitudinal shims (Z_2, Z_4) cause a non-symmetrical line shape. The higher the order (Z_4 is higher order than Z_2), the lower (closer to the base of the peaks) the problem is observed.



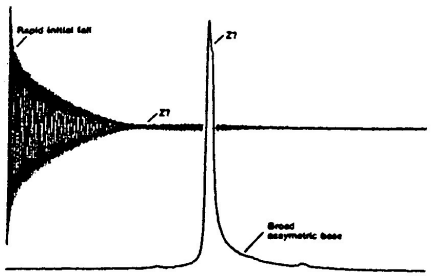
Z1 -- A "good" FID and a spectrum with small spinning sidebands are shown above in (a), but when the "Z" shim is changed (b), a characteristic beat in the FID is obtained, and the spectral line broadens and manifests structure that is an indicator of Z1 inhomogeneity.



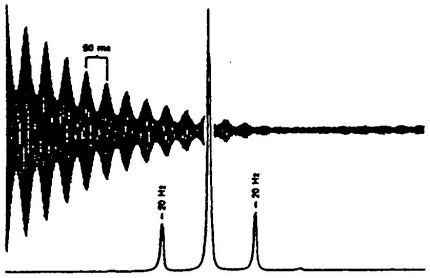
Z2 -- The asymmetric shape of the above peak (c) is typical of a mis-set Z2 shim. Note that the beats in the FID are less pronounced than in the diagram for Z1 (above, figure b) and that the initial descent is steeper. If the Z2 shim is mis-set in the opposite direction, then the asymmetry is reversed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



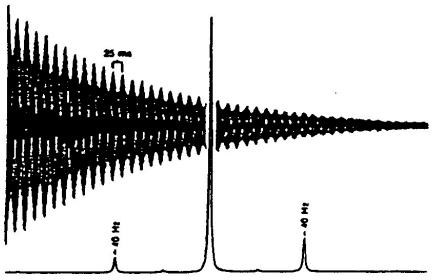
Z3 -- Although the broadened base of this peak is typical of a mis-set Z3 shim as is the rapid initial descent of the FID, there are also hints of +/-Z1 and +Z2 in the lineshape.



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, the only way to master shimming. That being said, knowledge of correct and incorrect 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.

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. Standard S/N measurements for ^1H spectra are always determined using a sample of 0.1 % ethylbenzene in $CDCl_3$ (ETB). A typical result for the Varian Inova 500 is 200:1 using the 5mm probe. It is important that the spectrum be acquired under the following standard conditions:

1. Use a 90 pulse.

2. Line Broadening of 1.0 Hz.

3. Spectral Width of 15 to 5 ppm.

4. A sufficient relaxation delay (at least $5xT_1$).

5. A sufficient digital resolution (less than 0.5 Hz/point).

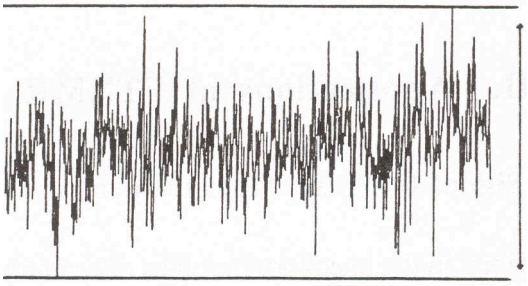
6. One transient.

Optimum signal-to-noise for any sample is defined as that which occurs when 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:

$$S/N = \frac{2.5A}{N_{pp}} \tag{5}$$

(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, and for 0.1 % ethylbenzene (ETB), use 5 to 3.5 ppm. 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 Varian instruments).

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 5% (w/v) menthol for ^{13}C, or when measuring routine ^{1}H survey 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 measuring 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 signals for any quaternary carbon atoms), you want to re-collect the spectrum to get an S/N of 50:1, a value more typical for ^{13}C NMR. Unfortunately, this will take 10 * l0 * 2 = 200 hours!

S/N Take Home Lesson

At some point, you may measure a spectrum and wonder why the signals are so weak. The majority 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. It is always a good practice to measure the spectrum of a standard, well-characterized compound before that of your unknown.

Integration

The purpose of this section of the handout is to show you how to obtain accurate integrals. The spectrum of 0.1 % ethylbenzene in CD_2C1_2 is given in Figure 7. $CDCl_3$ is not used in this case because the solvent peak overlaps with the phenyl region and complicates integration. If we assign an integral of 3.00 to the CH_3 triplet, then 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 15.6% too small, while the integral for the CH_2 quartet is off by only 3.5%. The 14.2% 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.

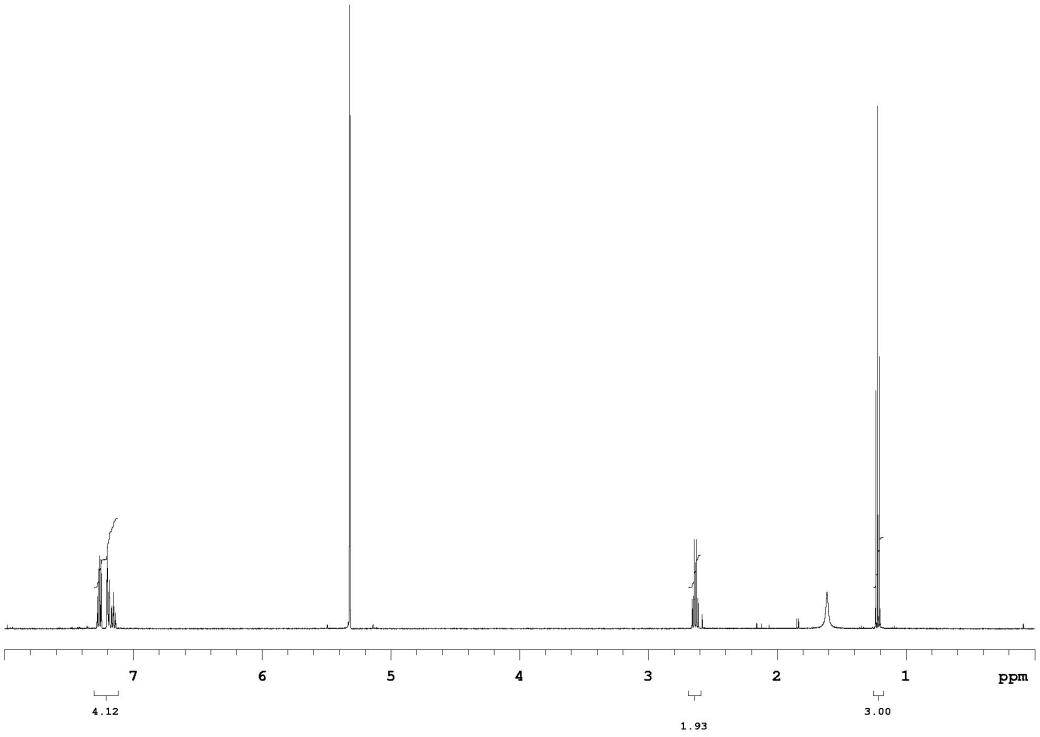


Figure 7. ^{1}H NMR spectrum of 0.1% ethylbenzene in CD_2Cl_2 taken on a Varian INOVA 500 MHz spectrometer with no recycle delay (nt=4, d1=0). The longest T_1 for this sample was measured at 12 seconds.

The accuracy of the integrals obtained for most routine spectra is usually about 10-20%. This accuracy is sometimes sufficient, especially if you already know what the compound is. However, this accuracy is usually not adequate to determine the exact number of hydrogen atoms 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 accuracy of 1-2%. For example, 20% accuracy is not sufficient to decide whether two peaks have a relative ratio of 1:3 or 1:4. Obtaining 1-2% accuracy

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 ends 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. When in doubt, it is best to be conservative. Hence if the peaks are separated by 3500 Hz, one can easily set the spectral width to 6000 – 8000 Hz. With modern spectrometers, this change puts an insignificant burden on the hardware.

III. The recycle time + the acquisition time = total time (TT) should be at least five T_1's. Data should be collected under conditions which ensure that all the nuclei can fully relax before the next FID is taken. The most challenging aspect of this criteria arises from three experimental variables. First, the acquisition time (**at**), should always match the sample. If the FID "rings" down quickly, a short value of **at** is fine. However, if the FID rings down slowly, then the value of **at** should be long enough such that it exceeds the last possible evidence of signal by about 50%. (You should routinely examine the time it takes the FID to ring down using the **df** (display FID) command). Next you need to consider how long T_1 may be with respect to the value of **at**. Note: when using a relatively long value **at**, with T_1 values that are not very long, means that you can use a relatively modest **d1** value and still enjoy nearly complete relaxation before the next pulse occurs. However, this is rarely the case. Hence, in contradistinction, in the case of 0.1 % ethylbenzene in CD_2C1_2, where the longest T_1 of interest is 9.8 sec (phenyl hydrogen atoms), so the TT when using a 90º pulse width should be 49 seconds. Even for a relatively long value of **at** (e.g. up to 6 – 10 seconds), a very long value of **d1** will be necessary to obtain accurate integrals.

IV. The spectrum should have a S/N of at least 250:1 for the smallest peak to be integrated. Usually 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. (Note: parameters in this group include **gain**, **sw**, **fb**, **rof2**, **alfa**, **lp** and **rp**.)

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.

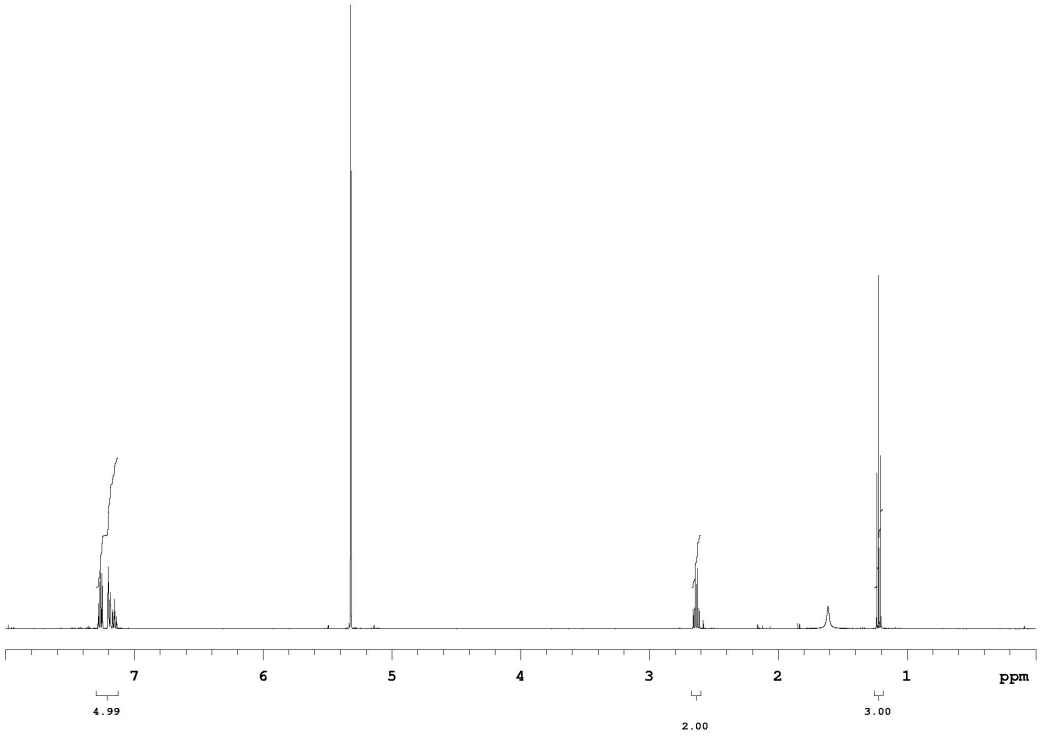


Figure 8. ^{1}H NMR spectrum of 0.1% ethylbenzene in CD_2Cl_2 taken on a Varian INOVA 500 MHz spectrometer with a recycle delay of 60 seconds (**nt=4**, **d1=60**). The longest T_1 for this sample was measured at 12 seconds.

With these points in mind, let's take the ^{1}H spectrum of ethylbenzene again. The major factor for poor integration in Figure 7 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 8 with only a 0.2% error of the aromatic protons.

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."

Homonuclear Decoupling

The purpose of this section of the handout is to explain what homonuclear decoupling does. Examples of a homonuclear decoupled spectrum are given in Figure 9. Homonuclear decoupling is a double-resonance technique that uses two Rf fields to affect magnetically active nuclei. Homonuclear decoupling involves applying a second RF field to cause selective saturation of nucleus A while observing all other nuclei in the

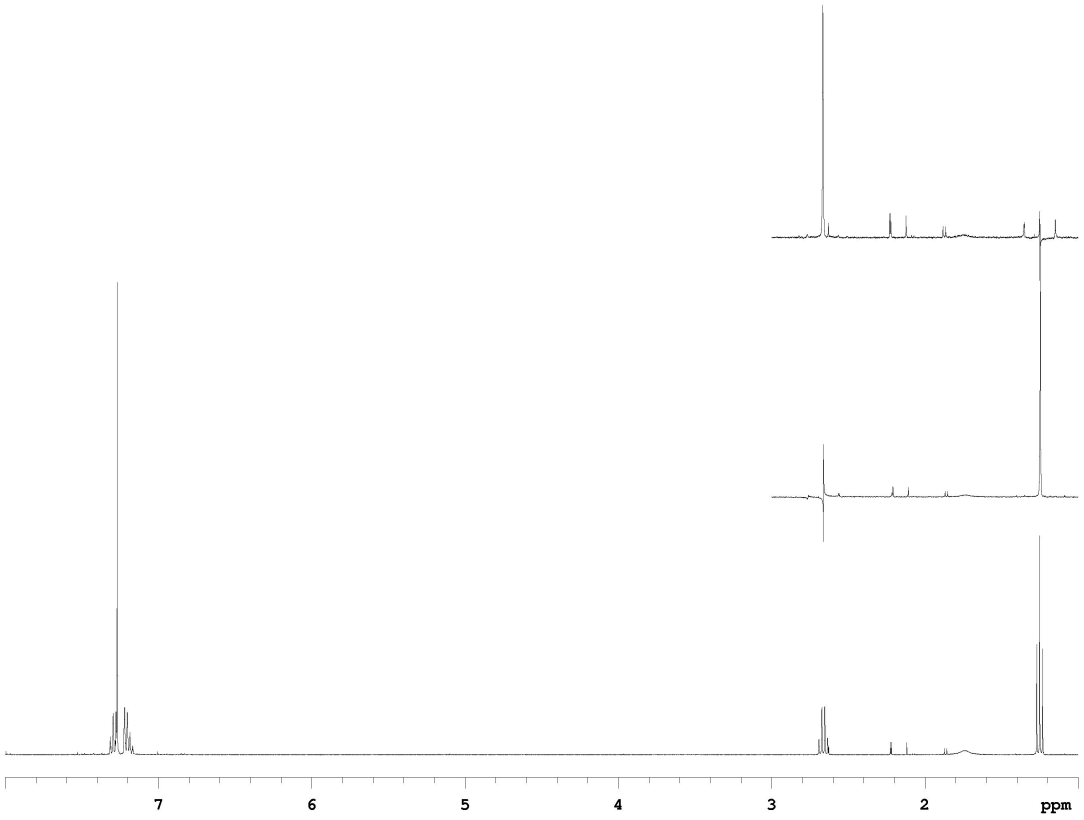


Figure 9. Proton spectrum of 0.1% ethylbenzene in $CDCl_3$ taken on a Varian Unity 400 MHz spectrometer. The lower trace is the full, coupled spectrum. The upper inset shows that by centering the decoupler on the triplet the quartet is collapsed to a singlet, while the lower inset demonstrates the effects of irradiating the quartet.

molecule; B, C, D, etc. If nucleus A is spin-coupled to nucleus B and if the second RF field is strong enough, the result is that A is effectively prevented from spin-spin interacting with B. The observed B nucleus spectrum will appear as if it is not coupled to A. The A resonance commonly appears as a glitch (*a.k.a. out of phase peak*) as a result of this experiment. As shown in Figure 9, if the triplet is homo-decoupled, the quartet collapses to a singlet. Similarly, if the quartet is homo-decoupled, the triplet collapses to a singlet. You may recall that a relatively high power, short Rf pulse will have a frequency spread due to the Heisenberg Uncertainty Principle; therefore, the second Rf field used for the selective decoupling will be lower power and have a longer duration.

As a general rule-of-thumb, you will need a second Rf field that has a frequency that is about 10 times larger than the coupling constant you are attempting to collapse. For example, if you what to collapse a 15 Hz coupling constant, you will need a B_1 field that is about 150 Hz in magnitude. This is precisely why understanding how to convert a 90° pulse width obtained at a particular value of **tpwr**, is essential to properly setting up a double-resonance experiment. (Note: exactly the same requirement exists when setting up a one-dimensional nOe experiment.) In this example, the soft irradiation on the single signal would be at a value of **tpwr** corresponding to 1/150 Hz = ~6700 μs for the 360° pulse-width, or about 1670 μs for the 90° pulse-width. Note: in practice to "deliver" this much power will sometimes require a modest increase in the setting of the value of **tpwr** (*typically about +5 – +10 dB*) because of the necessity of periodically interrupting the irradiation when each data point is recorded during the FID (*this interruption is called "time-shared-decoupling"*).

Homonuclear Decoupling Take Home Lesson

Homonuclear decoupling is a fast and effective way to establish that two nuclei are spin (scalar, 'J') coupled, and can be used to simplify a complex coupling pattern for further analysis. It is also useful as a follow-up to a COSY experiment to confirm specific couplings. To obtain definitive data the two signals should be separated by at least 0.5 ppm. It is also important to note that other signals close to the irradiation point may experience a displacement in their chemical shift due to the decoupling field. This displacement in the chemical shift is called a Bloch-Siegert shift and can be used to measure the decoupling field strength.

^{1}H 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 atoms are the NMR active isotope (1.10% are the NMR active ^{13}C), any means to improve S/N is essential. Splitting of the ^{13}C resonances as a result of coupling to attached hydrogen atoms will result in decreased S/N and is, thus, undesirable. Therefore, ^{13}C NMR spectra are typically run ^{1}H decoupled. The symbol ^{13}C-{^{1}H} is used to denote this and implies the ^{13}C nucleus is observed while the ^{1}H nuclei are being irradiated, thus decoupling them from the ^{13}C nuclei. A typical ^{13}C-{^{1}H} spectrum (5% menthol in acetone-d_6) is shown in Figure 10.

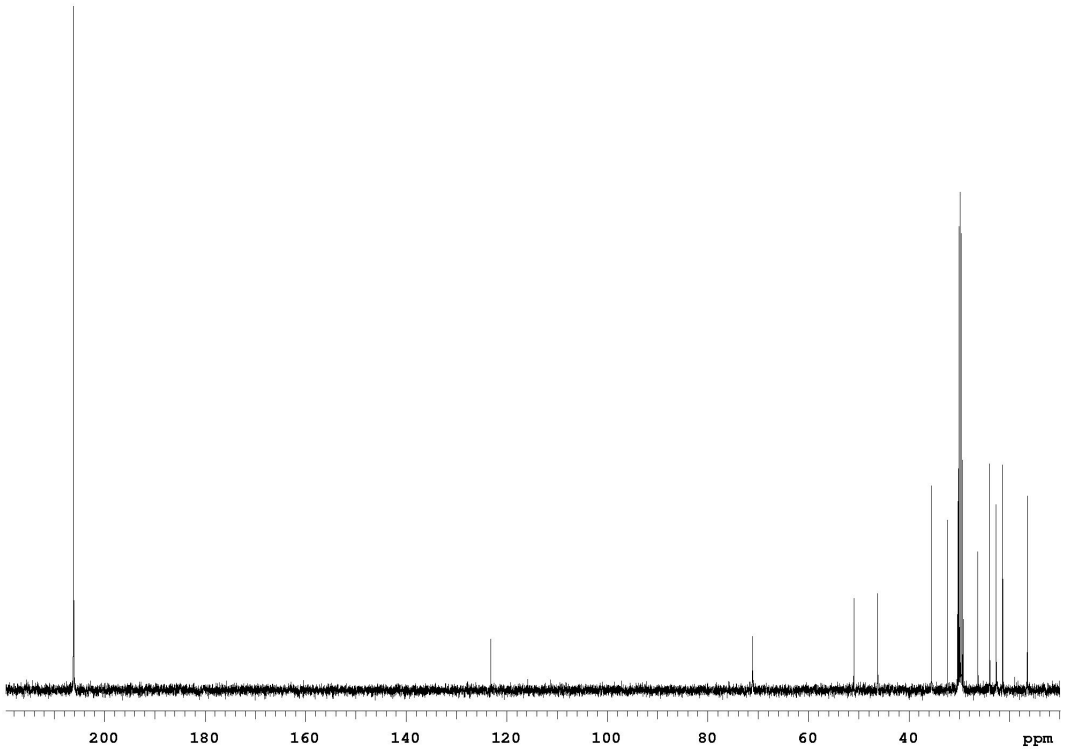


Figure 10. A ^{13}C-{^{1}H} NMR spectrum of a 5% solution of menthol in acetone-d_6 acquired on a Varian Unity 400 MHz spectrometer.

This is a double resonance experiment with the observed nucleus (^{13}C) and decoupled nucleus (^{1}H) on separate Rf channels. This experiment is called heteronuclear decoupling, and is another type of '1-PULSE' experiment, as described in the Basics section, with the addition of a decoupling field, as shown in Figure 11. It is the heteronuclear version of the homodecoupling experiment with the exception that broadband saturation (as opposed to selective saturation) is used.

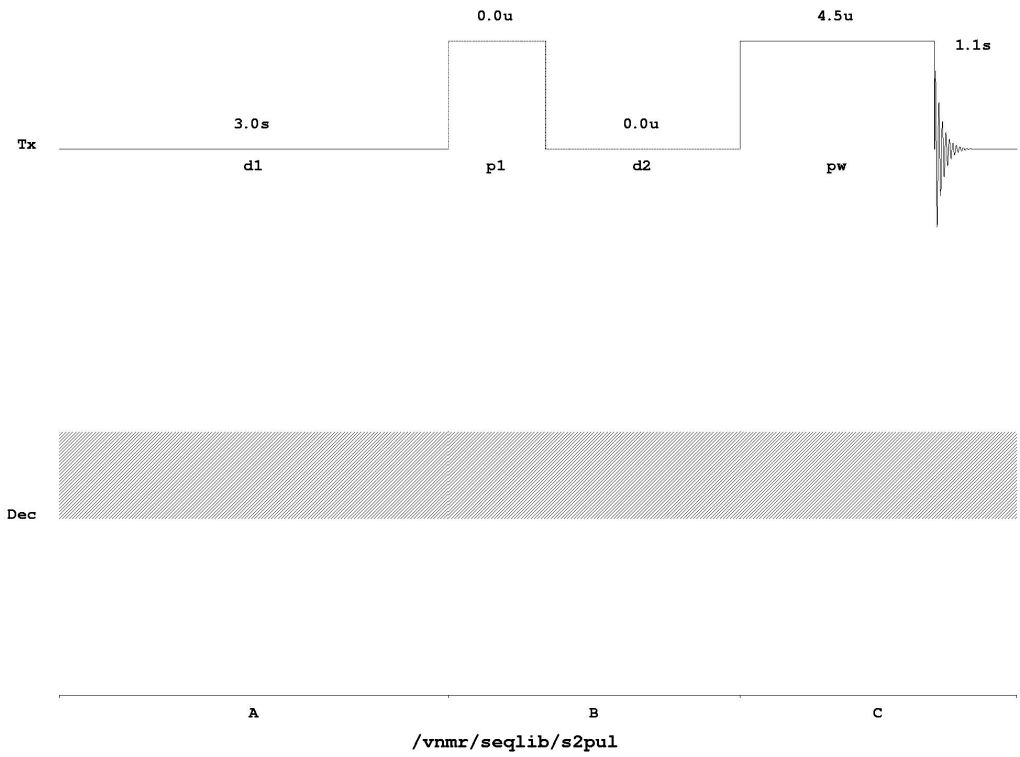


Figure 11. Representation of ^{13}C-{^{1}H}1-PULSE NMR experiment as presented on a Varian INOVA 500 MHz Spectrometer. Note that the **p1** pulse is unused in this sequence.

When acquiring spectra of nuclei other than ^{1}H (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 1-PULSE experiment is <u>not</u> quantitative, i.e., the integration of the peaks will not give a true indication of relative ratios because of a phenomenon called nuclear Overhauser enhancement (nOe) arising from the continuous broad-band saturation of the hydrogen atoms. ^{13}C nuclei that have directly bonded hydrogen atoms 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 ^{13}C and ^{1}H nuclei and is dependent on many factors. Thus, the nOe will be different for each unique carbon atom 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) ONLY during the acquisition time and gated off (turned off) during the recycle delay. This is shown in Figure 12.

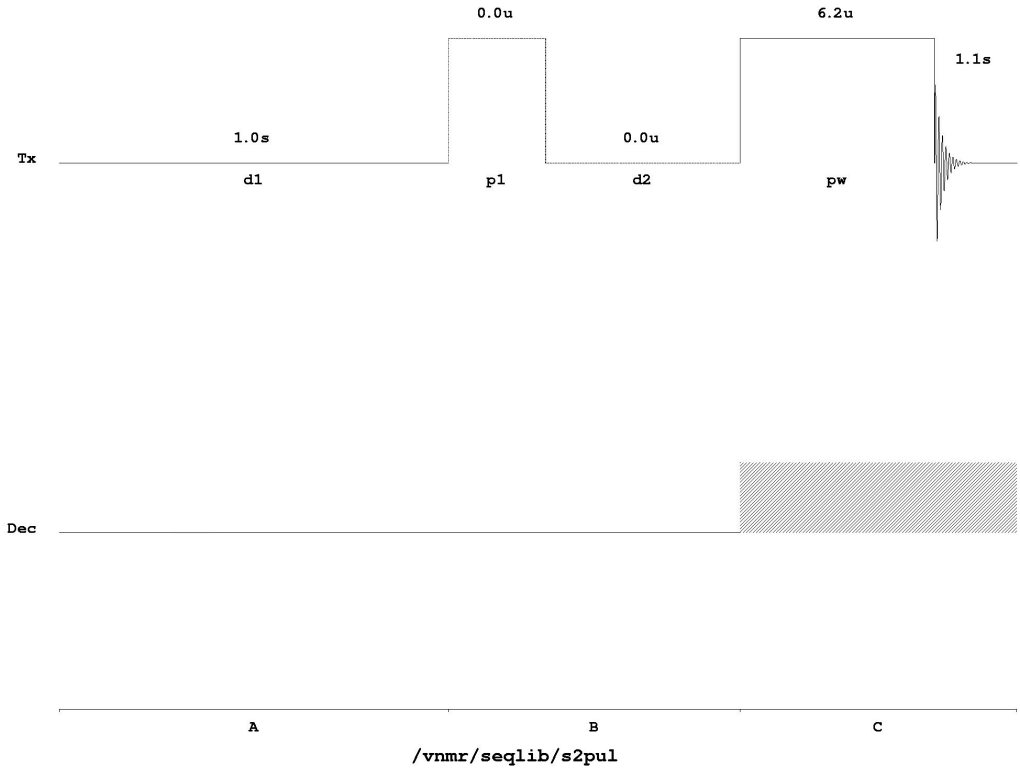


Figure 12. A gated decoupling pulse sequence for ^{13}C-{^{1}H} acquisition that has no nOe enhancement. Note that the decoupler channel (Dec) is only 'on' during segment C, which is the pulse and acquisition time. Compare to Figure 11.

The result of this experiment is a ^{13}C-{^{1}H} spectrum without NOE and is necessary for obtaining quantitative ^{13}C spectra. SPECIAL NOTE: gated decoupling typically requires an empirical increase in **d1**, to allow any nOe that might begin to accumulate during the later part of **at**, to dissipate fully before the next pulse.

II. **T_1 relaxation times:** The T_1's of ^{13}C nuclei are in general longer than those found for hydrogen atoms, as shown below in Figure 13. Therefore, you may have to wait very long times if you want accurate integrals from spectra. For example, from Figure 13, 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 carbon atoms have considerably longer T_1's and, as a result, typically have much smaller signals than other carbon atoms.

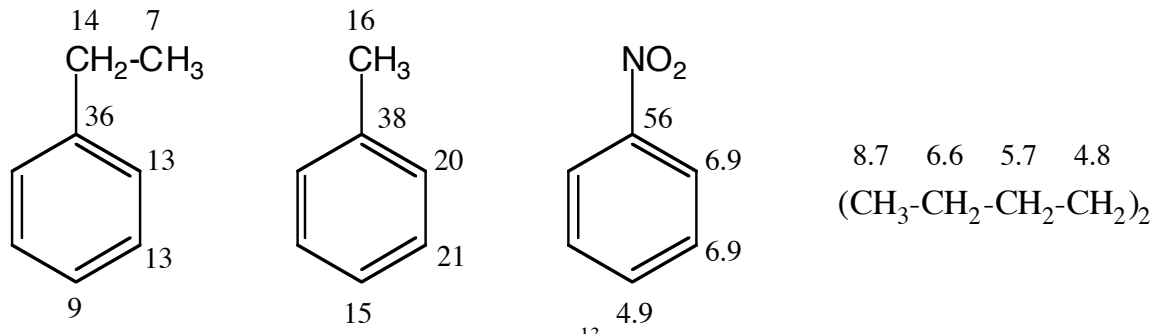


Figure 13. Examples of some representative ^{13}C NMR T_1 values, in seconds.

^{13}C NMR Take Home Lesson

Obtaining useful ^{13}C-{^{1}H} spectra requires knowledge of the same basics as needed for obtaining useful ^{1}H 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 ^{1}H to ^{13}C or other X nucleus, to increase the signal strength of the X nucleus. DEPT is an example of multi-pulse, multi-channel experiment, which uses synchronous pulses on two channels to afford polarization transfer. The pulse sequence is shown in Figure 14. In this case, the decoupler channel (Dec) is ^{1H} and the observe channel is ^{13}C. Since we are transferring the population difference of the hydrogen atoms to the X nucleus and gaining signal intensity from these hydrogen atoms, it is the ^{1}H T_1's that are important in determining repetition

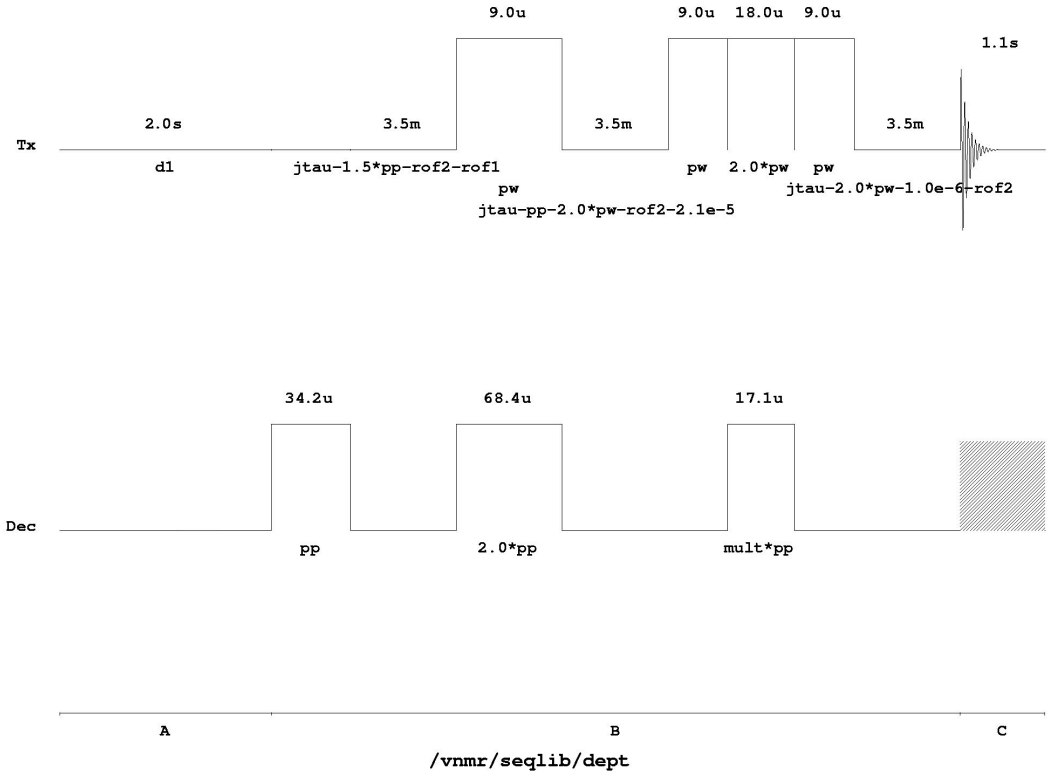


Figure 14. Schematic representation of a DEPT pulse sequence as displayed by a Varian INOVA 500 MHz spectrometer. The observe (Tx) channel is ^{13}C and the decoupler (Dec) channel is ^{1}H.

rate for a DEPT experiment. ^{1}H T_1's can be significantly shorter than that of ^{13}C 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 ^{1}H pulse (mult*pp) from 45 to 135º degrees, the multiplicity of the ^{13}C or X nucleus can be determined (i.e. depending on the pulse, the signal for a methine, methylene, or methyl carbon atoms will either be a positive, negative, or null signal, see table below). Remember, since quaternary carbon atoms have no attached hydrogen atoms, 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 15. Compare it to Figure 10. In general, you can run DEPT on most samples without additional calibration.

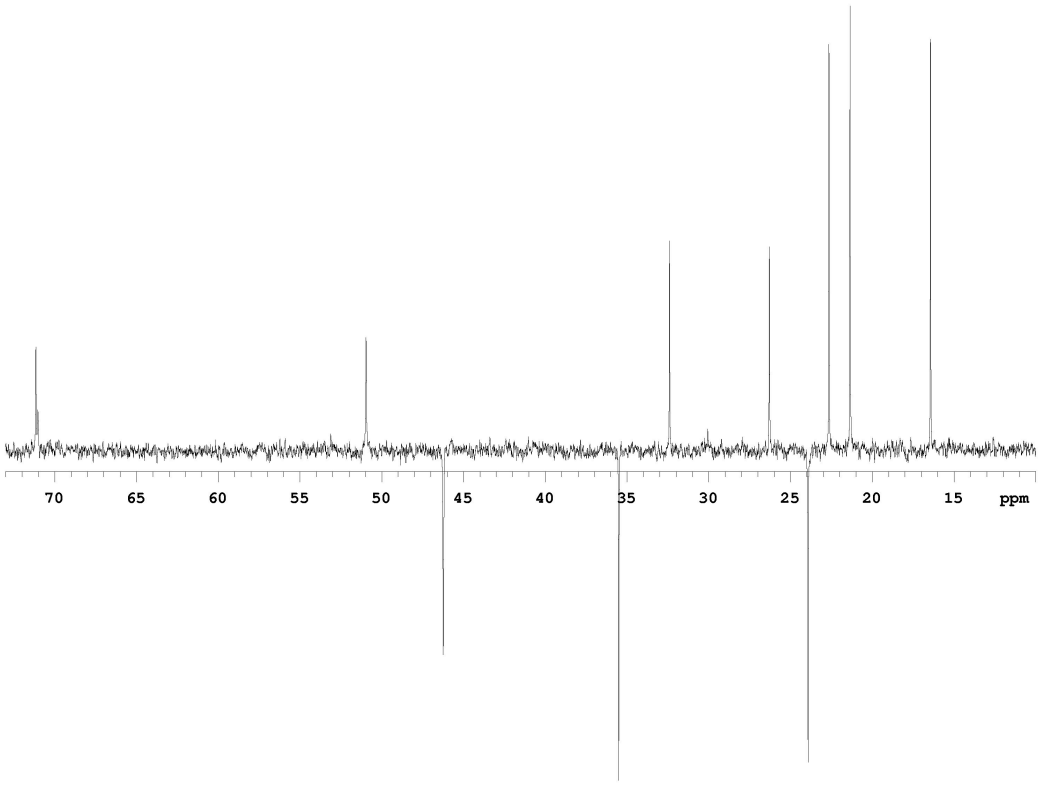


Figure 15. An example of a multiplicity-edited spectrum. Expansion of a ^{13}C-{^{1}H} DEPT-135 spectrum of menthol taken on a Varian Unity 400 MHz spectrometer. The relative sign of the peaks shows the multiplicity of the carbon atoms. The negative peaks are methylene carbon atoms, while the positive peaks are either methyl or methine carbon atoms (NOTE: quaternary carbon atoms are not detected).

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 carbon atoms.

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.