Basic NMR Concepts

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Note: This handout accompanies the NMR spectrometer training and checkout procedure.

I. Introduction

Now that you have attained a rudimentary understanding of NMR, you are ready to proceed to the next level checkout. The level 2 checkout requires an understanding of the basic NMR experiment as well as an understanding of basic instrument parameters. As compared to the level 1 checkout, you will now be able to use either menu mode or command mode to choose the desired parameters. Requirements for successful checkout are as follows:

1) Pass a written test on basic NMR principles
2) Be able to shim the magnet to meet linewidth at 1/2 height specification (0.5 Hz).
3) Be able to acquire proton spectra and carry out a homodecoupling experiment.
4) Be able to acquire proton-decoupled carbon spectra.
5) Be able to measure signal-to-noise (S/N) for a spectrum.
6) Be able to measure peak intensities by integration.

Specifically, the checkouts for the XL200 and QE300 involve collecting the following data:

(1) ^{1}H spectrum of 0.1 % Ethylbenzene/$CDCl_3$
Result: measurement of signal-to-noise; plot noise region

(2) ^{1}H spectrum of 0.1 % Ethylbenzene/$CDCl_3$
Result: measurement of spectrum with multiple scans; plot with integration

(3) ^{1}H spectrum of 0.1 % Ethylbenzene/$CDCl_3$ with homodecoupling
Result: measurement of spectrum after homodecoupling triplet and quartet; plot of both original and homodecoupled spectra.

(4) $^{13}C\{^{1}H\}$ spectrum of 57 % Menthol/$CDCl_3$
Result: measurement of $^{13}C\{^{1}H\}$ spectrum, S/N; plot spectrum and get peak printout.

(5) ^{1}H spectrum of $CHCl_3/d_6$-acetone
Result: measurement of shimming by determination of linewidth at half-height; plot expanded spectrum

The purpose of this handout is to teach you basic and applied information about FTNMR techniques. The information presented will help you pass the written test. **The purpose of the checkout is to teach you <u>how</u> to operate a spectrometer safely, while teaching you the necessary skills to acquire routine ^{1}H and ^{13}C data.** During the checkout you will insert/eject 3 samples, lock 3 samples, set up acquisition parameters for 5 operations, acquire 9 spectra, work up 9 data sets, and plot 5 spectra. More detailed instructions about how to carry out these operations are provided separately in handout #201 for the XL200 and handout #301 for the QE300.

II. Basics of FTNMR

The purpose of this section is to give you enough information about FTNMR experiments to avoid the most common errors. The handout will cover the most important parameters that affect any spectrum you may collect using an FTNMR spectrometer. The list of parameters is short:

Spectrometer Frequency
Pulse Width
Acquisition Time
Number of Points
Sweep Width
Relaxation Delay

The 1PULSE FTNMR experiment can be represented as shown in Figure 1.

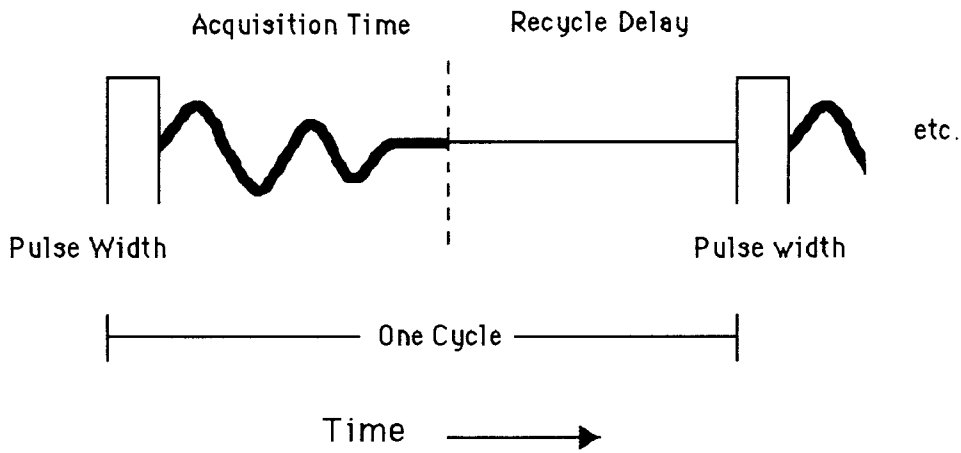


Figure 1

It is called a 1PULSE experiment because one radio frequency pulse is applied per cycle. The radio pulse excites the nuclei, which then re-radiate during the acquisition time, giving an NMR signal. The radio pulse has a characteristic frequency, called the spectrometer frequency (**SF**), which is dependent upon the nucleus you wish to observe and the magnetic field strength of the spectrometer you use. For protons, the spectrometer frequency on the QE300 is approximately

300.15 MHz, while on the XL200 the spectrometer frequency is approximately 200.06 MHz. The spectrometer frequency defines the center of the NMR spectrum you acquire.

The radio pulse also has a width, which is most commonly described in terms of a flip angle in degrees. As shown in Figure 2, this is because of its effect on the nuclear spins.

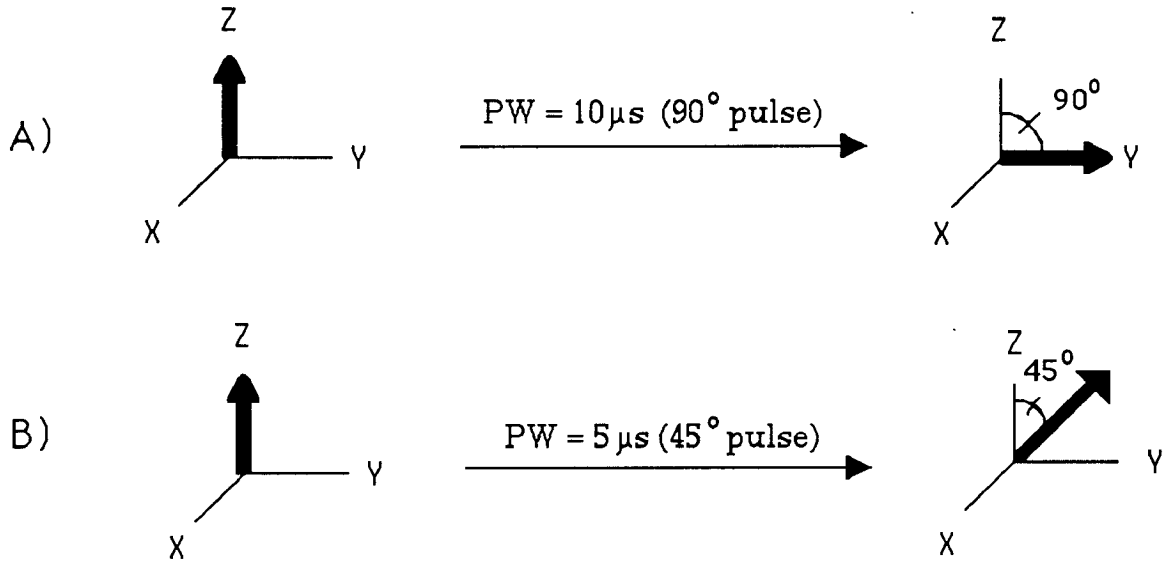


Figure 2 -- The average nuclear spin magnetization (heavy arrow) for an NMR sample placed in a magnetic field aligned along the Z-axis.

The 90 degree 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 90 degree pulse width for proton NMR experiments is about 10 μs on both the XL200 and the QE300. See handout #203 for the XL200 and handout #032 for the QE300). The pulse width is entered in microseconds by typing **PW** on the XL200 and **P2** on the QE300. The exact value is dependent upon the sample (nucleus, solvent, etc.) as well as the instrument (probe, etc.). For routine experiments, most users use a pulse width for their data collection that is less than the 90 degree pulse width. The reasons for this are discussed under relaxation delay.

So far, we have sent a pulse through the sample; now we need to acquire the resulting signal. In Figure 1, the decaying sine wave represents the Free Induction Decay or 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. The emitted radio intensity is digitized; in other words, the spectrometer represents the FID by a series of points along the FID curve. The number of points the spectrometer uses is referred to as the block size on the QE300 and can be changed by typing **CB**

for change block size; on the XL-200, the number of points can be changed by typing **NP**. In general, more points means higher resolution.

Fourier transforming the FID (commonly referred to as FT or FFT for Fast Fourier Transform) produces a spectrum which gives 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 (see page 1) and the spectral width or sweep width (referred to as **SW** -- see Figure 4).

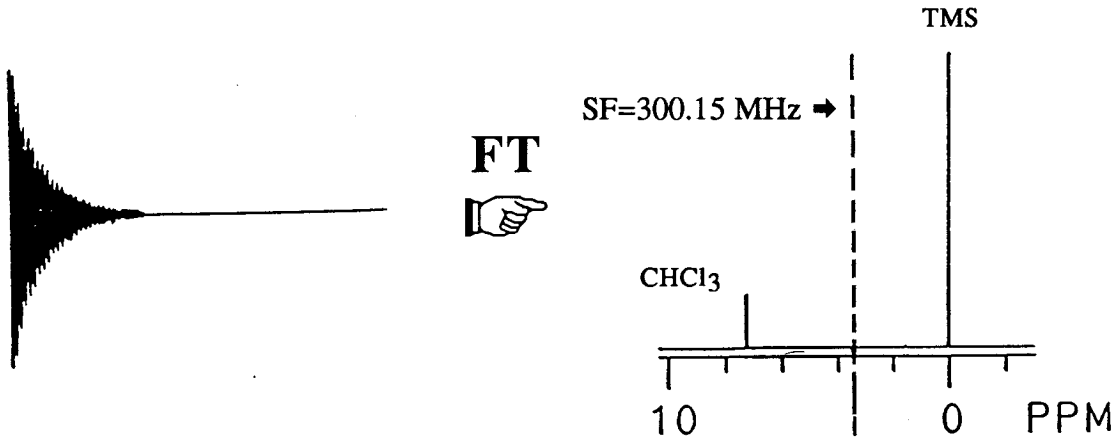


Figure 3 -- Fourier transform of the FID. Note: the spectrometer frequency you use, in general, will not be exactly 300.15 MHz.

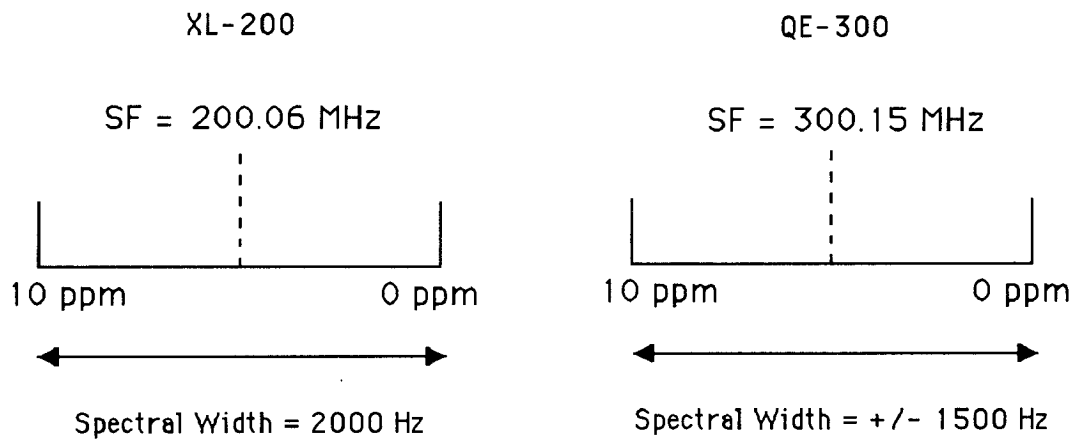


Figure 4

Example: At a spectrometer frequency of 300.15 MHz, a spectral width of approximately 3000 Hz is needed to scan 10 ppm (10 ppm x 300 Hz/ppm). At a spectrometer frequency of 200.06 MHz, a spectral width of approximately 2000 Hz is needed to scan 10 ppm (10 ppm x 200 Hz/ppm).

The spectral width is represented differently on the QE300 and XL200. On the XL200, the number you type in for **SW** is the total spectral width (the frequency difference between the left side and the right side of the spectrum), while on the QE300, the number you type in for **SW** is one-half the total spectral width (or the frequency difference between the center of the spectrum and one of the ends). For example, the spectral width for the 10 ppm wide spectra in the previous example would be typed in as SW=2000 Hz on the XL200, but as SW=+/- 1500 Hz on the QE300.

If SW, PT, and AT are defined as generic parameters, then they are related by the following equations, where SW = total spectral width, PT=number of points, and AT=acquisition time:

$$\mathbf{AT} = \frac{\mathbf{PT}}{\mathbf{2SW}} \qquad \text{Equation 1}$$

$$\mathbf{Resn} = \frac{\mathbf{1}}{\mathbf{AT}} = \frac{\mathbf{2SW}}{\mathbf{PT}} \qquad \text{Equation 2}$$

The digital resolution (Resn) 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 300.15 MHz, your total spectral width is 3000 Hz (10 ppm) and your required digital resolution (Resn) is 0.25 Hz/point, rearranging equation 2 gives you the number of points (PT):

$$PT = \frac{(2SW)}{Resn} = 24000 \text{ points}$$

Since the computer works most quickly if the number of points is a power of 2, the closest larger power of 2 would be used, 32768 points.

The spectral width, number of points, and acquisition time can be manipulated, but each instrument has different rules. On the XL200, the acquisition time can be entered directly from the

keyboard by typing **AT**; the XL200 will keep the spectral width constant and vary the number of points to effect the change. On the QE300, the acquisition time can not be changed directly. Instead, the digital resolution can be changed by typing **CB** and/or **SW** and entering new values for the block size and the spectral width. The QE300 will keep the number of points the same and then compute the acquisition time automatically.

The default ^1H values for the parameters discussed above are given below for the XL200 and QE300. Note that the standard parameters for the XL200 give a slightly better digital resolution (0.32 Hz/point) than for the QE300 (0.36 Hz/point). This merely reflects the difference in the default parameters for the total spectral widths and does not indicate the obtainable resolution for each instrument.

XL200	QE300
Spectral Width = 2600 Hz (13 ppm)	Spectral Width = +/- 3012 = 6024 Hz (20ppm)
Acquisition Time = 3.15 sec	Acquisition Time = 2.72 sec
Number of Points = 16384 points	Number of Points = 32768 points
Digital Resolution = 0.32 Hz/point	Digital Resolution = 0.36 Hz/point

We now have one last parameter to discuss, the recycle delay. On the XL200, this delay is named **D1**, while on the QE300, the delay is named **D5**. The total time between scans is given by the following equation, where TT is the total time, PW is the pulse width, AT is the acquisition time, and RD is the recycle delay: TT=PW+AT+RD (see Figure 1 for a pictorial representation).

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

$$\mathbf{TT=AT+RD}$$

The optimum recycle delay must be computed by rearranging the equation to give RD=TT-AT. The total time is important to ensure complete relaxation of the nuclear spins between scans; incomplete relaxation causes loss of signal and is one cause of inaccurate integrations. The relaxation time for a nucleus is called T1 (longitudinal relaxation time) and normally, when a 90 degree pulse width is used to excite the spins (Figure 2a), a total time (TT) of 5xT1 is necessary in order to have complete relaxation (see Figure 5). If a pulse width less than 90 degrees is used, the total time can be proportionally less.

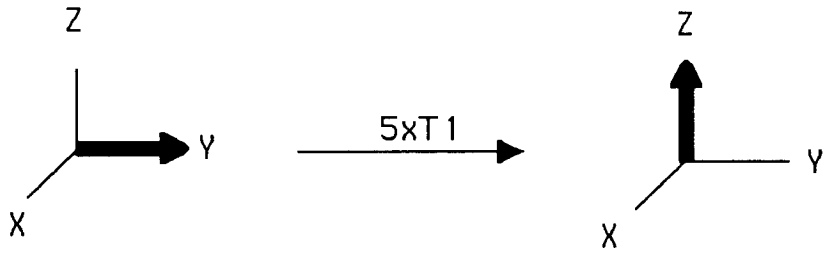


Figure 5

As an example of the above, if T1 is 600 msec, then the total time (where TT=5xT1) is 3.0s. The standard parameters given for the XL200 have the acquisition time set at 3.15 sec. As this is greater than 5xT1, the recycle delay (**D1**) could be set to 0 to satisfy the condition that the total time be greater than 5xT1. For the QE300, the acquisition time is set at 2.72 sec, which is less than 5xT1. Therefore, a recycle delay (**D5**) of 0.38 sec (3.0 sec - 2.72 sec) would be necessary to satisfy the condition. A discussion of how to measure T1's is given in handout #205 for the XL200 and #031B for the QE300, and a fuller discussion of how the choice of the spectral width and recycle delay affect integrations is given in section III.3.

Take Home Lesson

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

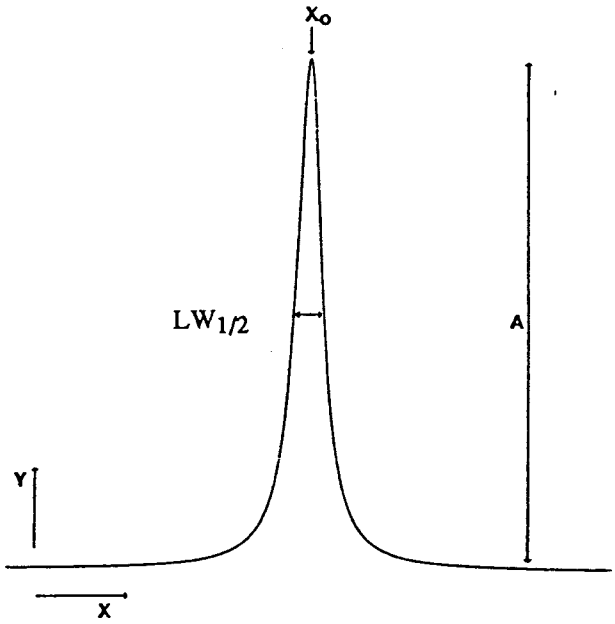
"Modern pulse NMR is performed exclusively in the Fourier transform mode. Of course it is useful to appreciate the advantages of the transform, and particularly the spectacular results which can be achieved by applying it in more than one dimension, but it is also essential to understand the limitations 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 FTNMR. 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."

III. Applications of FTNMR

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. Examples of reasonably well-shimmed spectra from the XL-200 and QE-300 are shown in Figures 1 and 2.

NMR peaks have a shape that is called Lorentzian. A Lorentzian line can be expressed mathematically and has three parameters (see below):



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

A = Amplitude
$LW_{1/2}$ = Peak width at half-height, in Hz
X_o = Peak position, in 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 ^1H 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. Lineshapes for ^1H NMR spectra are usually specified using $CHCl_3$ and the specifications are stated in terms of the peak width at 0.55% and 0.11% height of the $CHCl_3$ peak. (These percentages are chosen because they are the height of the ^{13}C satellites of the $CHCl_3$ line and one-fifth this height.) However, these values are meaningful only when compared with the half-height width ($LW_{1/2}$). From the mathematical equation for a Lorentzian line,, the linewidth at 0.55% height is calculated to be 13.5 times the $LW_{1/2}$, while the linewidth 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 linewidths at 0.55% and 0.11% height are very sensitive to how well shimmed the magnet is.

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.

(1) **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.

(2) **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.

(3) **Shimming problems are confirmed only if the "problem" is visible on every peak in your spectrum.** If 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.

(4) **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, then Z1. If you have problems shimming, ask MSL staff for help.

(5) **Spinning sidebands should always be below 2%.** If spinning sidebands are above 2%, turn off the spinner air, optimize x and y, 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 3. Although the peak in Figure 3b may have a line width at half-height ($LW_{1/2}$) that is less than 0.50 Hz, the checkout requirement, it is obviously poorly shimmed. You should never accept a poorly shimmed lineshape such as is shown in Figure 3b, 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. In general, odd-order shims (Z1, 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 (i.e., closer to the base of the peaks) the problem is observed.

Take Home Lesson

Knowledge of correct lineshapes allows you to decide quickly whether your sample is correctly shimmed. You have to decide whether the return (a better lineshape) is worth the time spent achieving that lineshape.

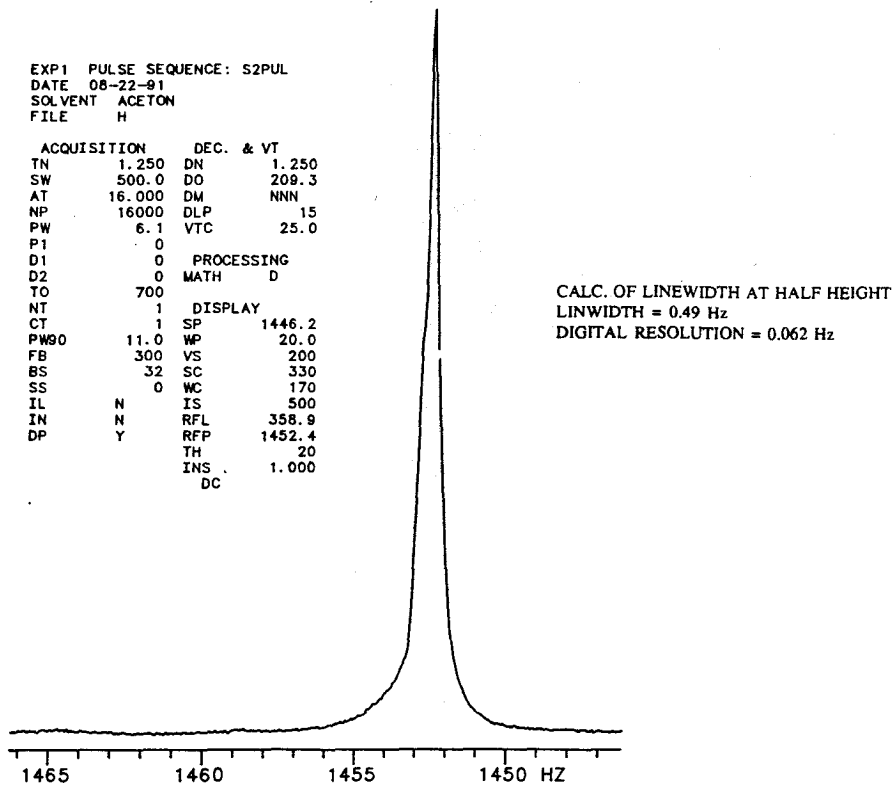


Figure 1: Typical $CHCl_3$ peakwidth at half-height obtained on the XL200.

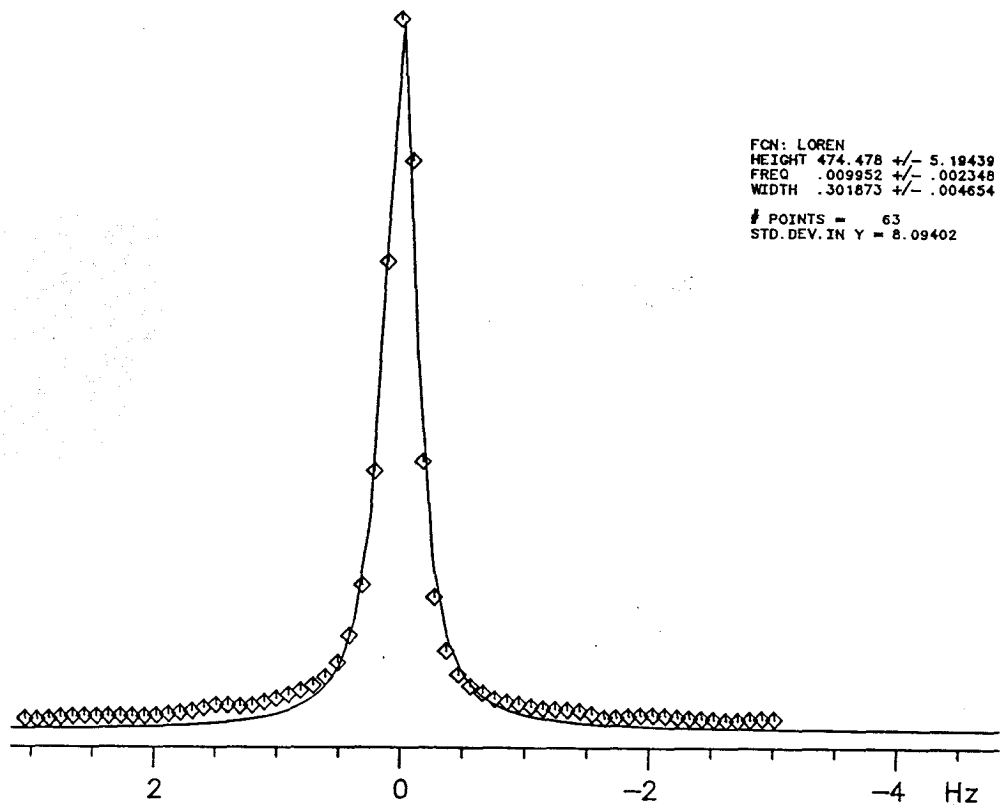
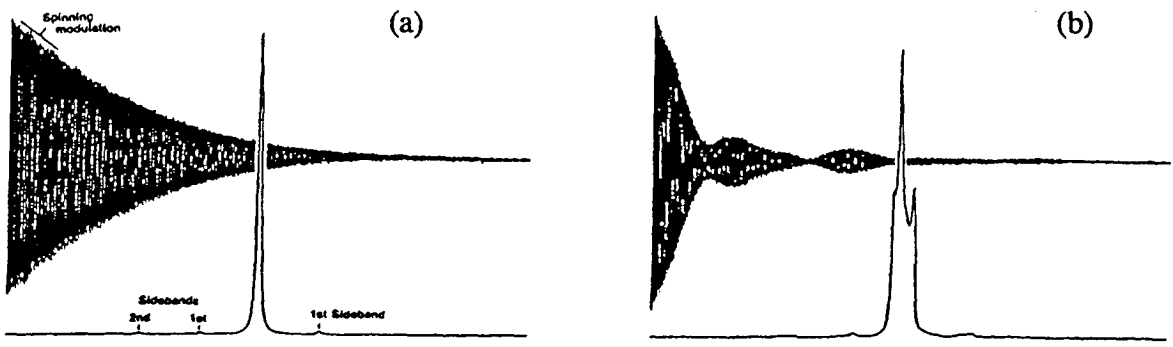
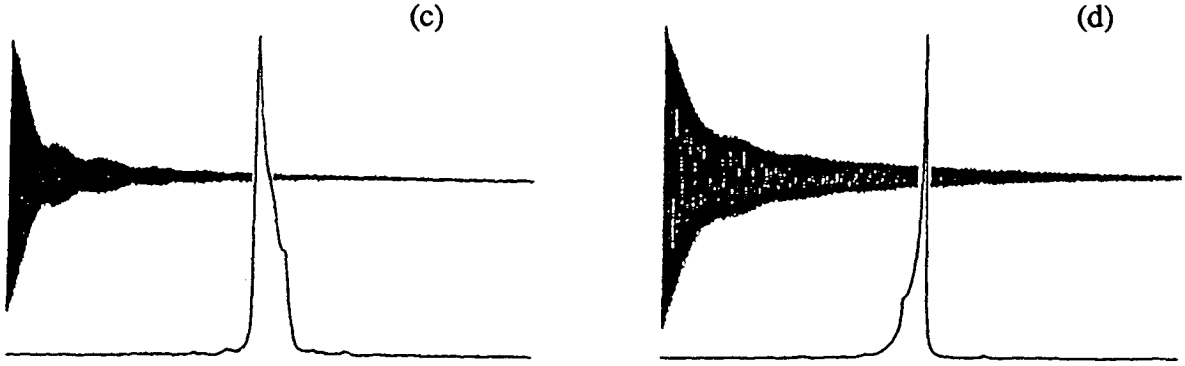


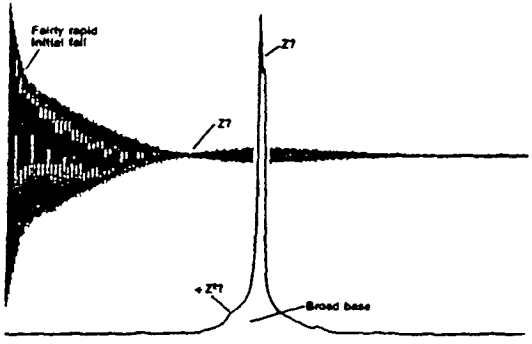
Figure 2: Typical $CHCl_3$ peakwidth at half-height obtained on the QE300.



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.

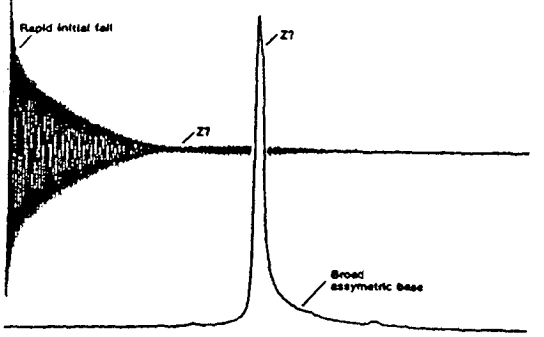


Z2 -- The asymmetric shape of the above peak (c) is typical of a mis-set Z2 shim. Note that the beats in the FID are less pronounced than in the diagram for Z1 (above, figure b) and that the initial descent is steeper. If the Z2 shim is mis-set in the opposite direction, then the asymmetry is reversed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

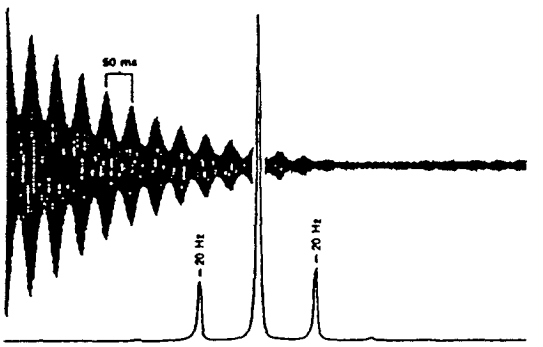


Z3 -- Although the broadened base of this peak is typical of a mis-set Z3 shim as is the rapid initial descent of the FID, there are also hints of +/-Z1 and +Z2 in the lineshape.

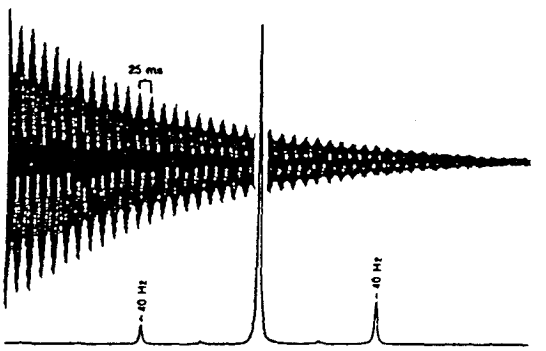
Figure 3: Taken from Gwendolyn Chmurny and David Hoult, "The Ancient and Honourable Art of Shimming." In Concepts in Magnetic Resonance. (1990). Volume 2: pp. 131-149.



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 ($\mathbb{R}^2$) -- 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 3 (continued): Taken from Gwendolyn Chmurny and David Hoult, "The Ancient and Honourable Art of Shimming." In Concepts in Magnetic Resonance. (1990). Volume 2: pp. 131-149.

III. Applications of FTNMR

2) Signal-to-Noise Measurement

The first spectrum described in the checkout involves determining a signal-to-noise measurement, or S/N. S/N is an important criterion for accurate integrations (see section 3), and is also one of the best ways to determine how sensitive an NMR spectrometer is. In general, a higher S/N specification means that the instrument is more sensitive.

A typical result for the XL200 checkout is S/N ≅ 20:1 and is shown in Figure 1, while a typical result for the QE300 checkout is S/N ≅ 70:1 and is shown in Figure 2. If you measure a S/N for the ETB standard that is significantly less than these values, write your observations in the spectrometer notebook and inform the MSL staff.

S/N measurements for proton spectra are always determined using a sample of 0.1 % ethylbenzene in $CDCl_3$ (ETB). It is important that the spectrum be acquired under standard conditions:

1. 90° pulse width
2. Line Broadening = 1.0 Hz
3. Spectral Width = 15 to 5 ppm
4. A sufficient relaxation delay (at least 5xT1)
5. A sufficient digital resolution (less than 0.5 Hz/point)
6. One acquisition

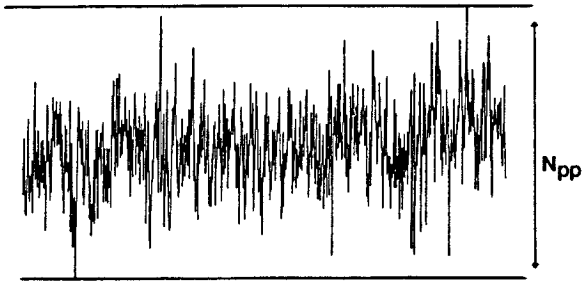
Optimum signal-to-noise for any sample is achieved using a line broadening equal to the peak width at one-half height. When this line broadening is applied, the peak width at half-height doubles, i.e., it is the sum of the natural peak width at one-half height plus the line broadening applied.

The equation used for calculating S/N is :

$$S/N = \frac{2.5xA}{N_{pp}}$$

A = height of the chosen peak
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 occur with low probability, so there seems to be a large gap between them and what appears to be the main part of the noise; will-power must be exercised in order to truly measure this gap and not to dismiss it in our minds as "spikes" rather than the incoherent noise it is. True instrumental defects resulting in spikes can be dismissed, but only after several measurements have proven a spike is always present.



Choice of a noise region must be consistently applied for standard samples, and for 0.1% ethylbenzene (ETB), we have chosen 5 to 3.5 ppm. It is also crtitical that the noise be amplified if necessary in order to make an accurate measurement using a ruler. There is not much point in trying to compare the ratio of noise a few millimeters high to a peak 20 cm high; plot the noise such that it is several centimeters high. If the instrument performs the signal-to-noise measurement and calculation, the relative intensities are not as critical.

The last point concerning sensitivity is demonstrated during the $^{13}C\{^{1}H\}$ part of the checkout. The checkout requires that you acquire four spectra, using 1, 4, 16, and 64 acquisitions. Typical S/N values obtained are:

NA	XL200	QE300
1	10	25
4	20	50
16	40	100
64	80	200

Note: Signal-to-noise increases as the square root of the number of acquisitions; 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 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 $^{13}C\{^{1}H\}$ spectrum on the QE300 for 2 hours, you get peaks with a 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 recollect the spectrum to get a S/N of 50:1, a value more typical for carbon NMR. Unfortunately, this will take 10x10x2 = 200 hours! In such cases, you should consider using the Unity 400 or GN500 instruments, which will be able to give you a spectrum with good S/N in much less time.

Take Home Lesson

At some point, you may take a spectrum and wonder why the signals are so weak. Well over 75% 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 ^{1}H (or ^{13}C) standard such as ETB (or menthol). In this way, you can save yourself needless frustration on the instruments by identifying problems that are due to a bad sample.

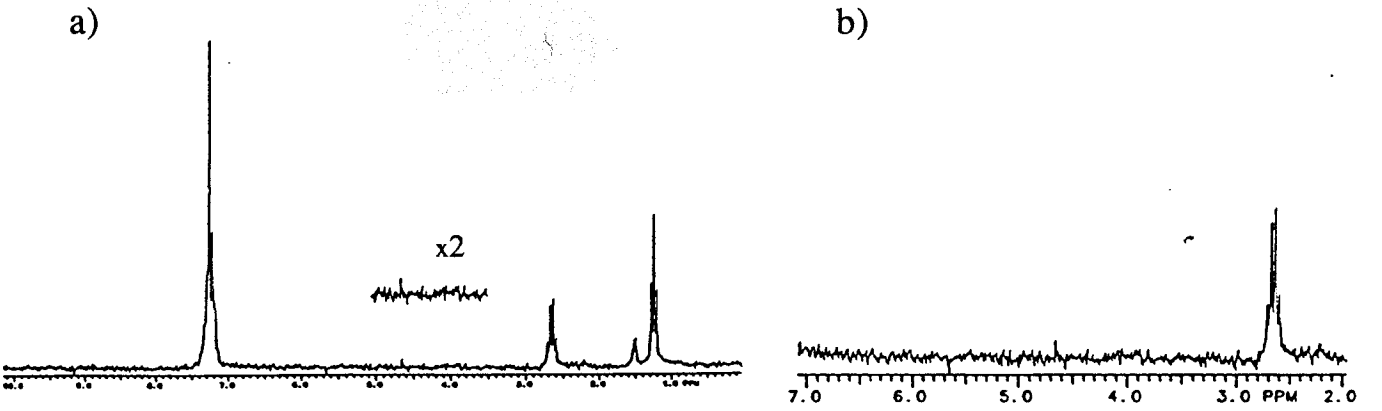


Figure 1: ^1H spectrum of 0.1% ethylbenzene/$CDCl_3$ at 200.06 MHz, using standard parameters for S/N determination. (a) Full spectrum with 3.5 to 5.0 ppm region multiplied by a vertical expansion factor of two. (b) Plot of 2.0 to 7.0 ppm region.

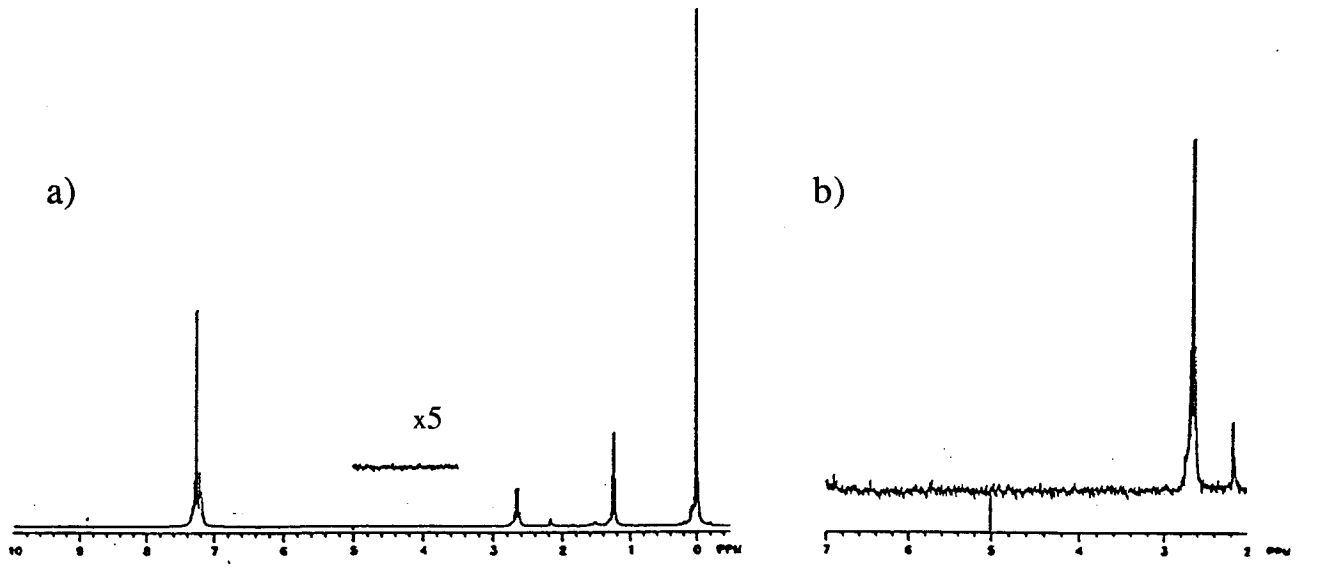


Figure 2: ^1H spectrum of 0.1% ethylbenzene/$CDCl_3$ at 300.15 MHz, using standard parameters for S/N determination. (a) Full spectrum with 3.5 to 5.0 ppm region multiplied by a vertical expansion factor of five. (b) Plot of 2.0 to 7.0 ppm region.

III. Applications of FTNMR

3) Integration

The purpose of this section of the handout is to show you how to obtain accurate integrals. Examples of spectra of 0.1% ethylbenzene in $CDCl_3$ from the XL200 and QE300 are given in Figures 1 and 2, respectively. Both spectra were taken using the default parameters for acquiring ^1H spectra.

In both spectra, there is a problem with getting an accurate integration for the phenyl region due to the overlapping peak for the residual protons in $CDCl_3$. This problem can be solved by switching to another solvent, CD_2Cl_2; the resulting spectrum, also acquired with default parameters, is shown in Figure 3 (from the QE300). If we assign an integral of 3.00 to the CH_3 triplet, then the phenyl region integrates to 4.29 protons, while the CH_2 quartet integrates to 1.97 protons. Thus, the integral for the phenyl protons is 14.2% too small, while the integral for the CH_2 quartet is off by only 1.5%. 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.

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 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 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.

1) **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.
2) **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 300.15 MHz, if two peaks are separated by 7 ppm, a spectral width of at least 2100 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, 210 Hz, giving a total spectral width of about 2520 Hz (8.4ppm). Although the standard setup parameters on the XL200 and QE300 should easily satisfy this criterion, you should be prepared to make the spectral width larger if necessary.

3) **The recycle time should be at least five T1'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 90 degree pulse widths are used, relaxation delays of 5xT1 of the longest T1 of interest are necessary. The results of a quick experiment to determine the the T1's of 0.1% ethylbenzene in CD_2Cl_2 is shown in Figure 4. (For detailed information on finding 90 degree pulse widths, see handout #203 for the XL200 and handout #032 for the QE300; for detailed information on T1 studies, see handout #205 for the XL200 and handout #031B for the QE300). Note the difference in T1 between the phenyl and alkyl protons, and the residual protons in CD_2Cl_2. The longest T1 of interest is 9.8 sec for the phenyl protons, so the relaxation delay when using a 90° pulse width should be 49 sec (5x9.8). In Figure 5a is shown the spectrum of 0.1% ethylbenzene/CD_2Cl_2, using a 90 degree pulse width, a relaxation delay of 49 seconds, taking 32 acquisitions, and using a line broadening of 0.1 Hz (for comparison, the default parameters on the QE300 use a 50° flip angle and a recycle delay of 3.2 sec). If we assign an integral of 3.00 to the CH_3 triplet, then the phenyl region integrates to 4.91 protons, while the CH_2 quartet integrates to 1.92 protons. Thus, the integral for the phenyl protons is now only 1.8% too small, while the integral for the CH_2 quartet is off by 4.0%. The errors for both the phenyl protons and the CH_2 protons are now comparable; they reflect a good choice for the recycle delay for this sample.

4) **The spectrum should have a S/N of at least 250:1** for the smallest peak to be integrated. The S/N measured for the quartet in Figure 5a is 245:1, which is close to fulfilling this criterion. We can improve this number by choosing a line broadening that is approximately equal to the true (natural) linewidths of the peaks. For this ethylbenzene sample, the lines are approximately 1 Hz in width. Figure 5b shows the same data as in Figure 5a, but processed using an optimal line broadening of 1.0 Hz. The S/N measured for the quartet is now 464:1. With the CH_3 triplet in Figure 5b assigned an integral value of 3.00, the phenyl region integrates to 4.98 protons and the CH_2 quartet integrates to 1.95 protons. The integral for the phenyl protons is now 0.4% too small while the integral for the CH_2 quartet is off by 2.5%. The line broadening of 1.0 Hz is optimal for this sample, and gives better integrals because the S/N increases. Usually if you cannot see any baseline noise, you probably have close to the required S/N for accurate integrals.

5) **The baseline should be flat**. Distortion due to phase problems should be corrected. Baseline distortion due to non-optimum parameter selection causing a baseline roll are not discussed here. See lab staff for help if you suspect this problem.
6) **The peaks need to be sufficiently digitized**, as discussed in the BASICS section of this handout. If the linewidth at half-height is 1 Hz, you need a digital resolution of less than 0.5 Hz.
7) **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.

A summary of the integrals presented for Figures 2, 5a, and 5b is given in Table 1. The reason the integral for the phenyl region is so inaccurate for the spectrum in Figure 2 is because the recycle delay was too short. Obviously, if you did not know the phenyl region represented 5 instead of 4 protons, you could draw an erroneous conclusion about the structure of this compound from the integrals in Figure 2.

Table 1

Spectrum	I_{CH3}	I_{Phenyl}	%error	I_{CH2}	%error
Figure 2	3.00	4.29	14.2	1.97	1.5
Figure 5a	3.00	4.91	1.8	1.92	4.0
Figure 5b	3.00	4.98	0.4	1.95	2.5

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 FTNMR, 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."

Region	Integral
Phenyl	6.8
Quartet	1.7
Triplet	3.0

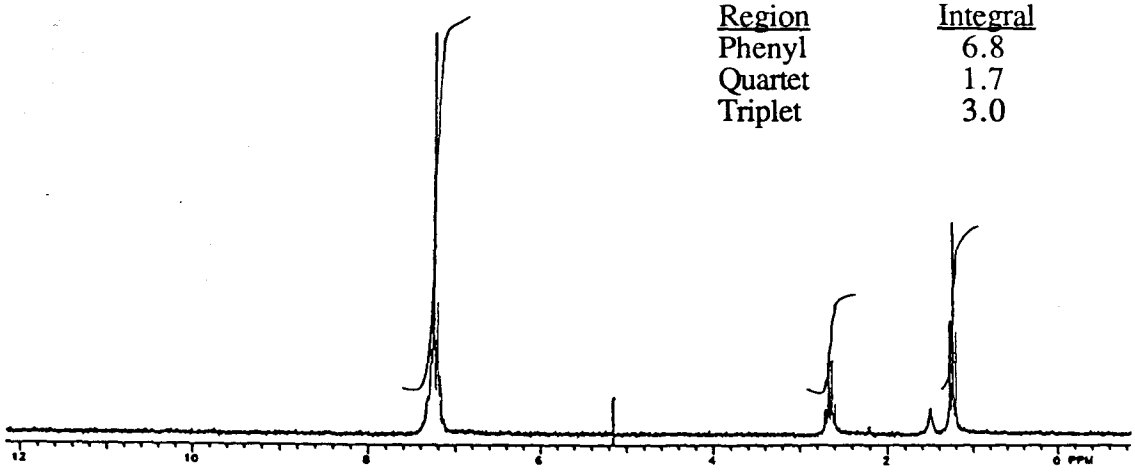


Figure 1: ^1H spectrum of 0.1% ethylbenzene/$CDCl_3$ at 200.06 MHz, using default ^1H parameters, 32 acquisitions, and a line broadening of 0.1 Hz.

Region	Integral
Phenyl	6.4
Quartet	2.0
Triplet	3.0

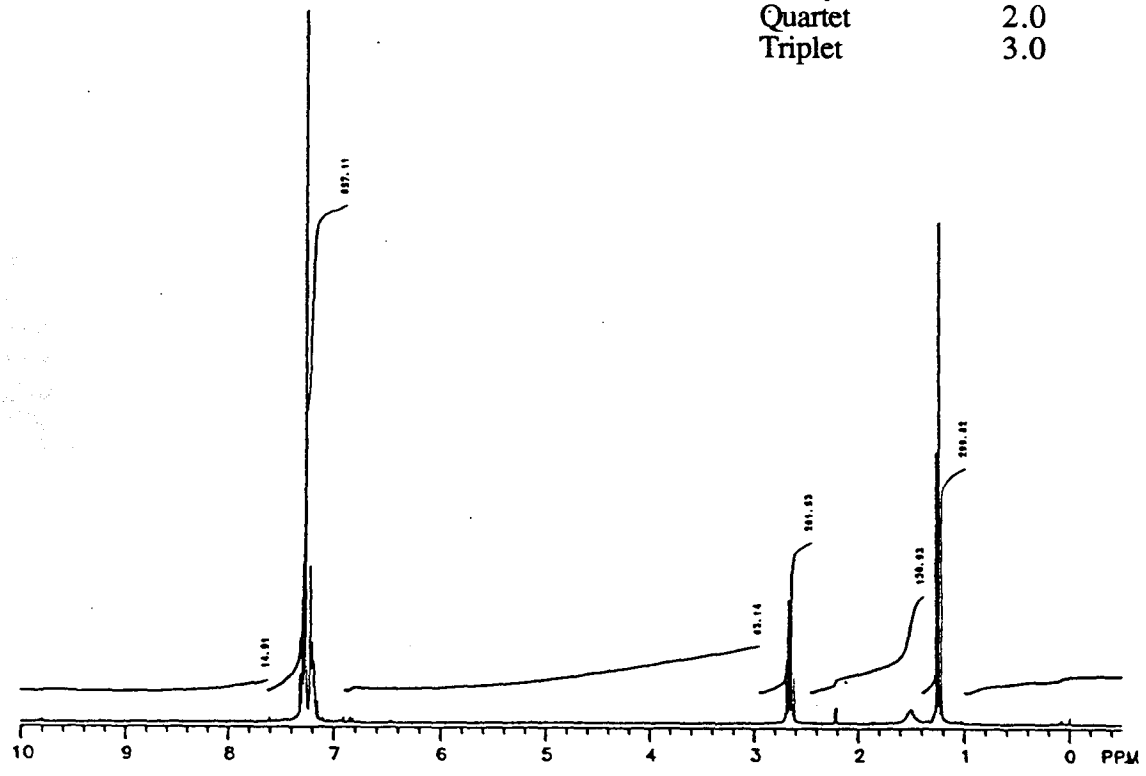


Figure 2: ^1H spectrum of 0.1% ethylbenzene/$CDCl_3$ at 300.15 MHz, using default ^1H parameters, 32 acquisitions, and a line broadening of 0.1 Hz.

Region	Integral
Phenyl	4.3
Quartet	2.0
Triplet	3.0

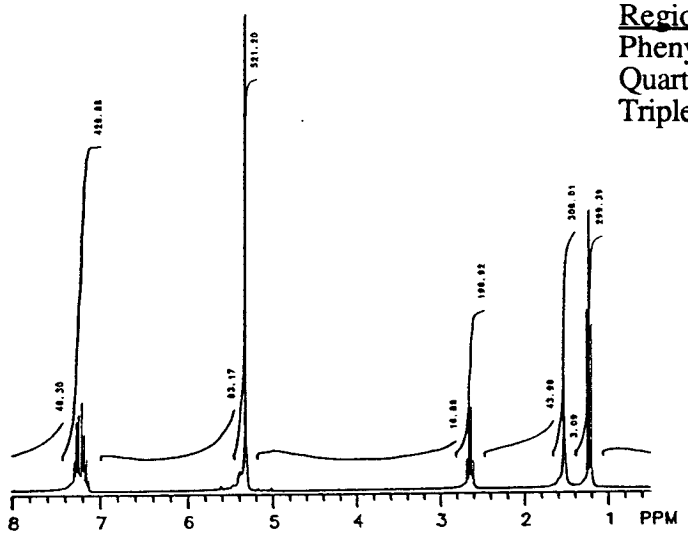


Figure 3: ^1H spectrum of 0.1% ethylbenzene/CD_2Cl_2 at 300.15 MHz, using default ^1H parameters, 32 acquisitions, and a line broadening of 0.1 Hz.

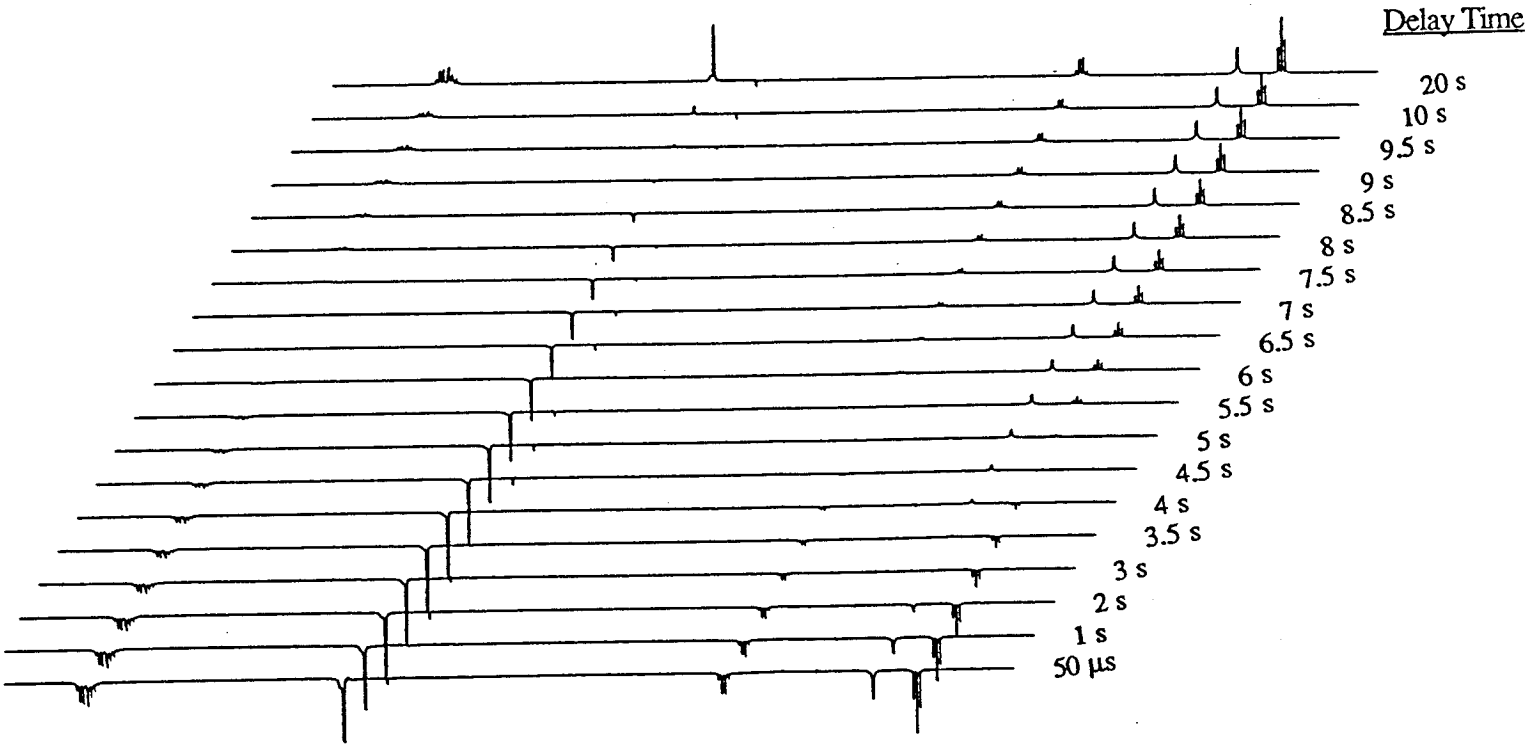
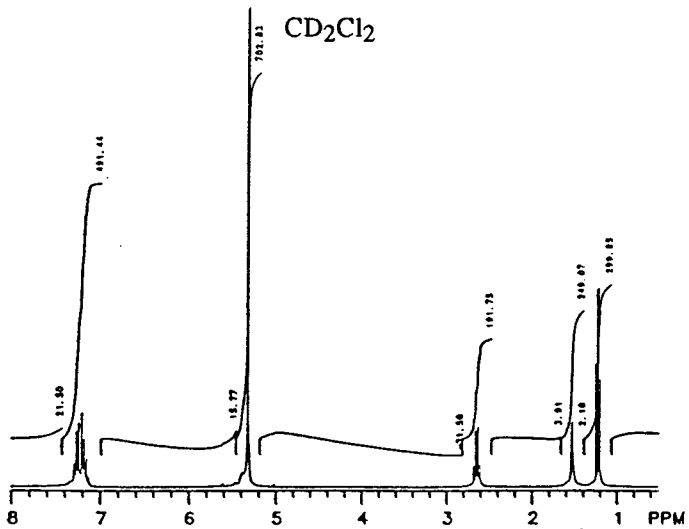


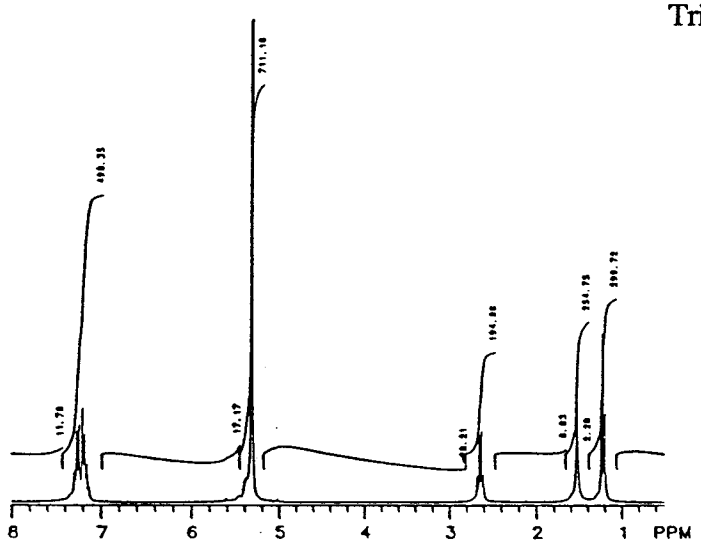
Figure 4: T1 study of 0.1% ethylbenzene/CD_2Cl_2 by the inversion recovery method. The delay time which results in an intensity of zero for a particular signal is called that signal's "null time." The T1 can be estimated by dividing the null time by 0.69 (ln2).

(a)



Region	Integral
Phenyl	4.9
Quartet	1.9
Triplet	3.0

(b)



Region	Integral
Phenyl	5.0
Quartet	2.0
Triplet	3.0

Figure 5: ^1H spectrum of 0.1% ethylbenzene/CD_2Cl_2 at 300.15 MHz, taking 32 acquisitions, using a 90° flip angle and a recycle delay of 5xT1 (longest).
a) Line broadening is 0.1 Hz
b) Line broadening is 1.0 Hz

III. Applications of FTNMR

4) Homo-decoupling

The purpose of this section of the handout is to explain what homo-decoupling does. Examples of homo-decoupled spectra taken on the XL200 and QE300 are given in Figures 1 and 2, respectively.

Homo-decoupling is a double-resonance technique because it uses two RF fields to affect magnetically active nuclei. Homo-decoupling involves applying a second ^1H RF field to cause selective saturation of a nucleus A while observing all other nuclei in the 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 as a result of this experiment.

Note: In Figures 1 and 2, 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.

Take Home Lesson

Homo-decoupling is an effective way to establish that two nuclei are spin coupled, and to simplify a complex coupling pattern for further analysis. It can be difficult to obtain definitive data if the two nuclei are closer than 0.5 ppm to each other.

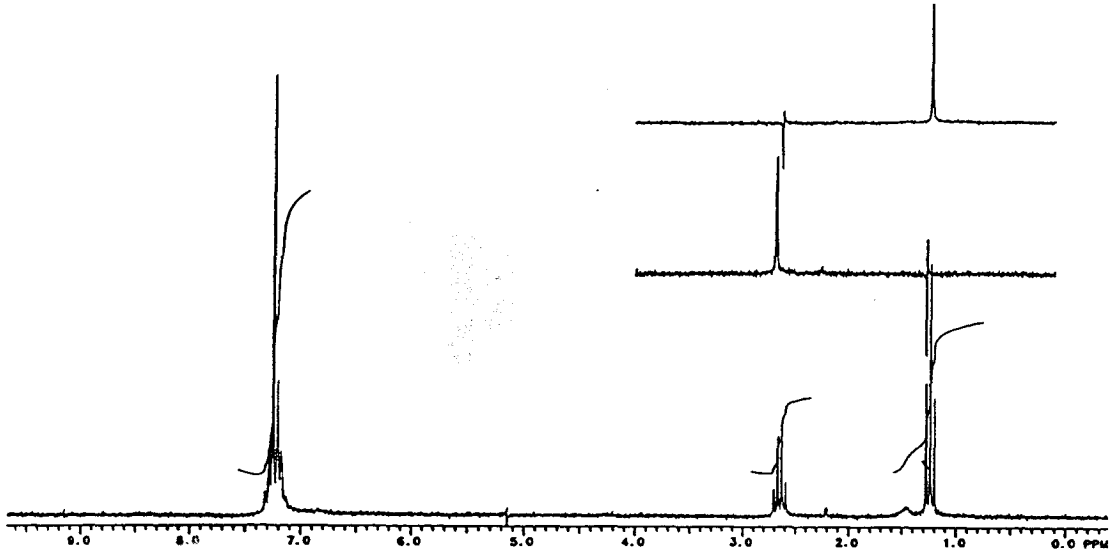


Figure 1: ^{1}H spectrum of 0.1% ethylbenzene/$CDCl_3$ at 200.06 MHz, using default ^{1}H parameters, 32 acquisitions, and homo-decoupling at the indicated sites. The upper inset plot is the result of homo-decoupling the triplet, while the lower inset plot is the result of homo-decoupling the quartet. The full-sized plot is the normal spectrum.

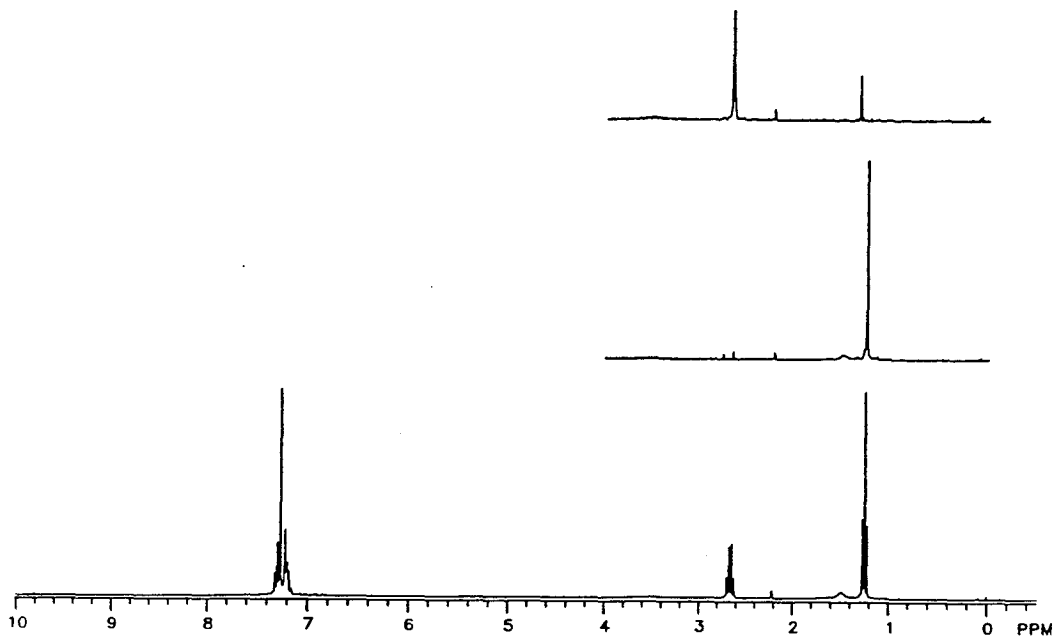


Figure 2: ^{1}H spectrum of 0.1% ethylbenzene/$CDCl_3$ at 300.15 MHz, using default ^{1}H parameters, 32 acquisitions, and homo-decoupling at the indicated sites. The upper inset plot is the result of homo-decoupling the quartet, while the lower inset plot is the result of homo-decoupling the triplet. The full-sized plot is the normal spectrum.

III. Applications of FTNMR

5) $^{13}C\{^{1}H\}$ Spectra

The purpose of this section of the handout is to give you some useful information about $^{13}C\{^{1}H\}$ spectroscopy. Examples of spectra of 57% menthol in $CDCl_3$ taken on the XL200 and QE300 are given in Figures 1 and 2, respectively. Some of the pertinent default ^{13}C values for the XL200 and QE300 are given below.

XL200	QE300
Spectrometer Frequency = 50.3 MHz	Spectrometer Frequency = 75.6 MHz
Spectral Width = 14000 Hz	Spectral Width = 20000 Hz
Acquisition Time = 2.14 sec	Acquisition Time = 0.82 sec
Number of Points = 60000 (zero filled to 65536)	Number of Points = 32768
Digital Resolution = 0.21 Hz/point	Digital Resolution = 0.61 Hz/point
Line Broadening = 1.0 Hz	Line Broadening = 1.0 Hz

To begin, the symbol $^{13}C\{^{1}H\}$ implies a ^{13}C spectrum where the ^{1}H nuclei are decoupled from the ^{13}C nuclei. This is a double resonance experiment, just as described in section 3 for homodecoupling, except that now the observed nucleus (^{13}C) and decoupled nucleus (^{1}H) are not the same. This experiment is called heterodecoupling (or heteronuclear decoupling), and is a 1PULSE experiment, as described in the Basics section, with the addition of the decoupling field (Figure 3).

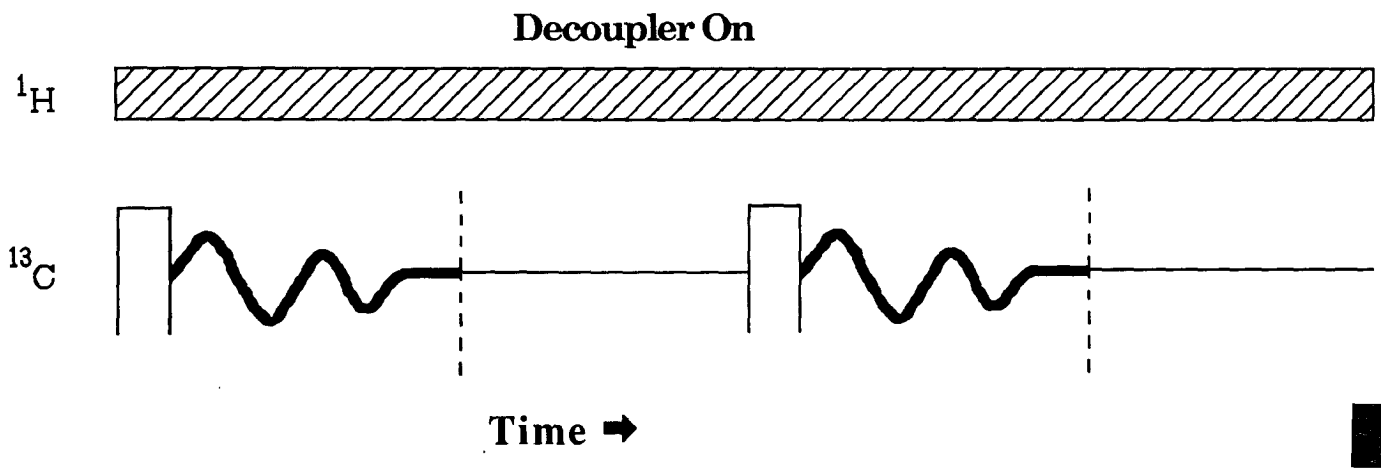


Figure 3 -- Representation of $^{13}C\{^{1}H\}$ 1PULSE experiment.

The $^{13}C\{^{1}H\}$ spectrum obtained using a standard 1PULSE experiment is not quantitative, i.e., the integration of the peaks will not give a true indication of relative ratios because of the nuclear Overhauser enhancement (nOe) of the ^{13}C nuclei due to their attached ^{1}H nuclei. For the case of ^{13}C spectra acquired

with proton decoupling, an enhancement of up to 1.98 (i.e., 198%), or an almost threefold improvement in signal-to-noise is expected for those carbon nuclei that are attached to protons.

The digital resolution given by the default ^{1}H and ^{13}C parameters on the XL200 and QE300 differ by a factor of three. This is mainly due to the need for a larger spectral width on the QE300 to accomodate the larger chemical shift range for ^{13}C and to the need to use fewer points to conserve space on the hard disk. Due to the large sweep widths typical of $^{13}C\{^{1}H\}$ spectra, it is important that the number of points not be too small, or distortions of your peaks can occur. Figure 4 shows a spectrum of 57% menthol/$CDCl_3$ collected with only 8192 points at 75.48 MHz. Compare it to Figure 2 and note the anomalous peak heights in Figure 4, as well as the phase distortion in the peaks around 30ppm. These effects are due to an insufficient number of data points and is not an instrument problem. The distortion can be eliminated by increasing the number of points from 8192 to 32768.

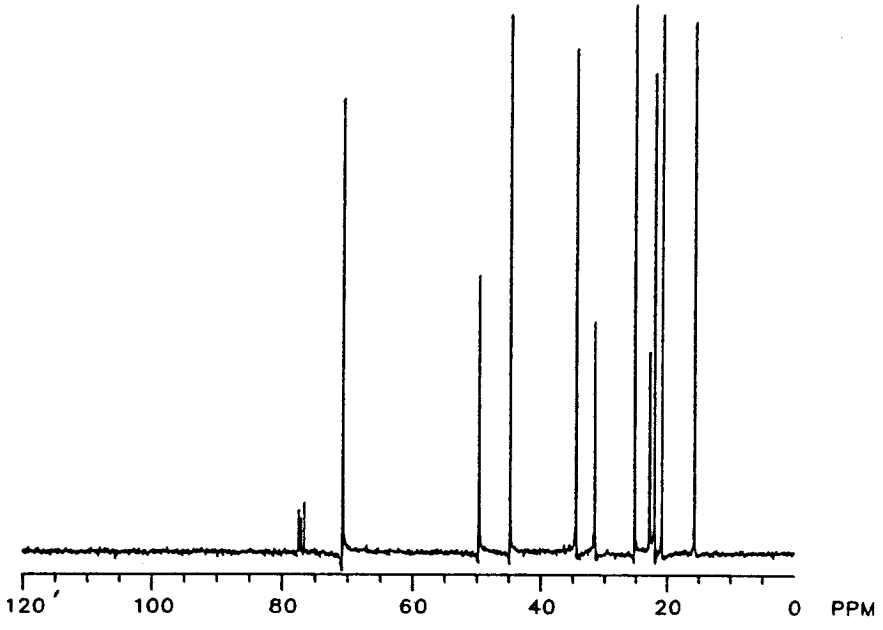
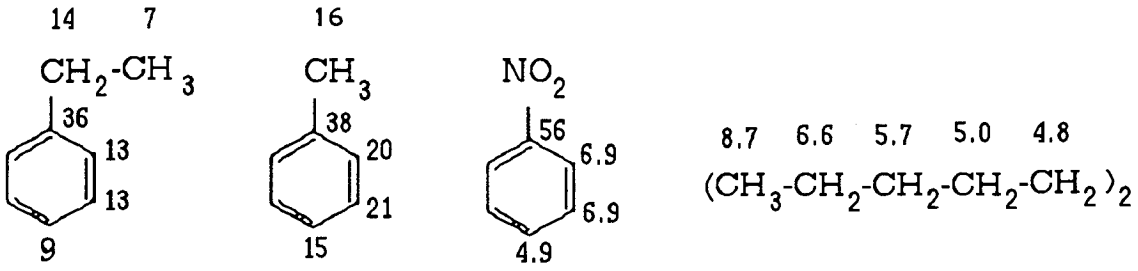


Figure 4: $^{13}C\{^{1}H\}$ spectrum of 57% menthol/$CDCl_3$ at 75.6 MHz using default parameters, **except 8192 points** (default is 32768 points).

Integration in $^{13}C\{^{1}H\}$ NMR Spectroscopy: To obtain quantitative $^{13}C\{^{1}H\}$ spectra, you must do two things: follow the protocol given in section 3 on integration and carry out a gated decoupling experiment, i.e., the decoupler is gated on (turned on) during the acquisition time and gated off (turned off) during the recycle delay. A T1 study of 90% ethylbenzene/$CDCl_3$ is shown in Figure 5, with the results summarized below. Note: The T1's of ^{13}C nuclei are in general much longer than those found for ^{1}H nuclei. Therefore, you may have to wait very long times if you want accurate integrals from spectra. A paramagnetic relaxation agent such as $Cr(acac)_3$ (available from Aldrich) can be used to shorten the T1's, but can sometimes be difficult to separate from the compound afterwards.

Examples of T1's for some ^{13}C nuclei are given below (all numbers are in seconds):



(see figure 4)

The 1PULSE experiment shown in Figure 3 can be altered to suppress the nOe, as shown in Figure 6, so that ^{1}H decoupling is on only during the acquisition time. This is called a gated experiment, as the decoupler is gated on only during the acquisition time. The result of this experiment is a $^{13}C\{^{1}H\}$ spectrum without nOe. These two cases are compared in Figure 7, with the results summarized in Table 1. See lab staff if you wish to perform the gated experiment.

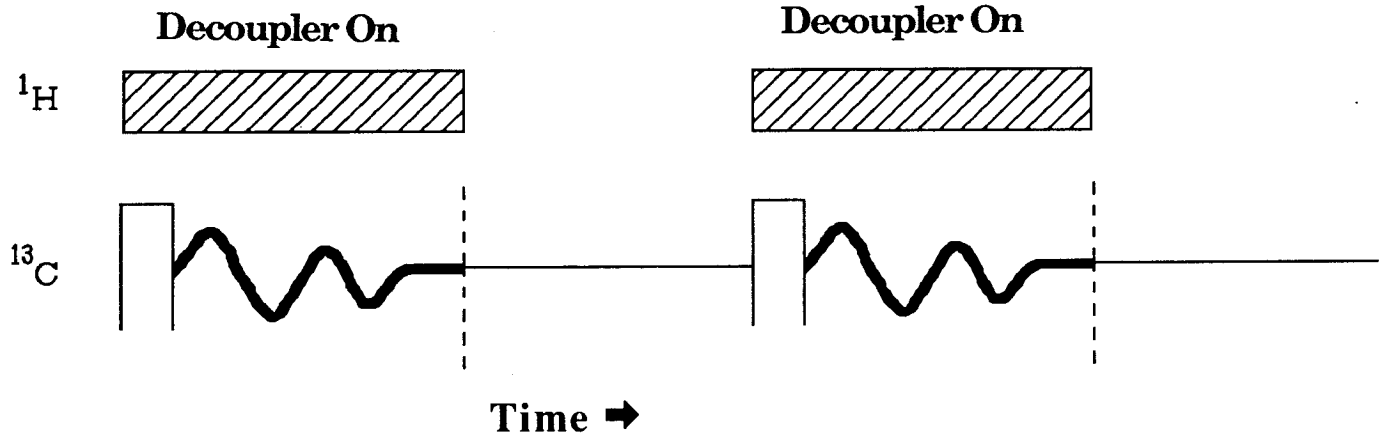


Figure 6 -- Representation of $^{13}C\{^{1}H\}$ - Decoupler Gated On During Acquisition Time Experiment.

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 yourself needless frustration on the instrument by taking a quick spectrum of a ^{13}C standard and checking the S/N, or seeing if the standard is decoupled properly.

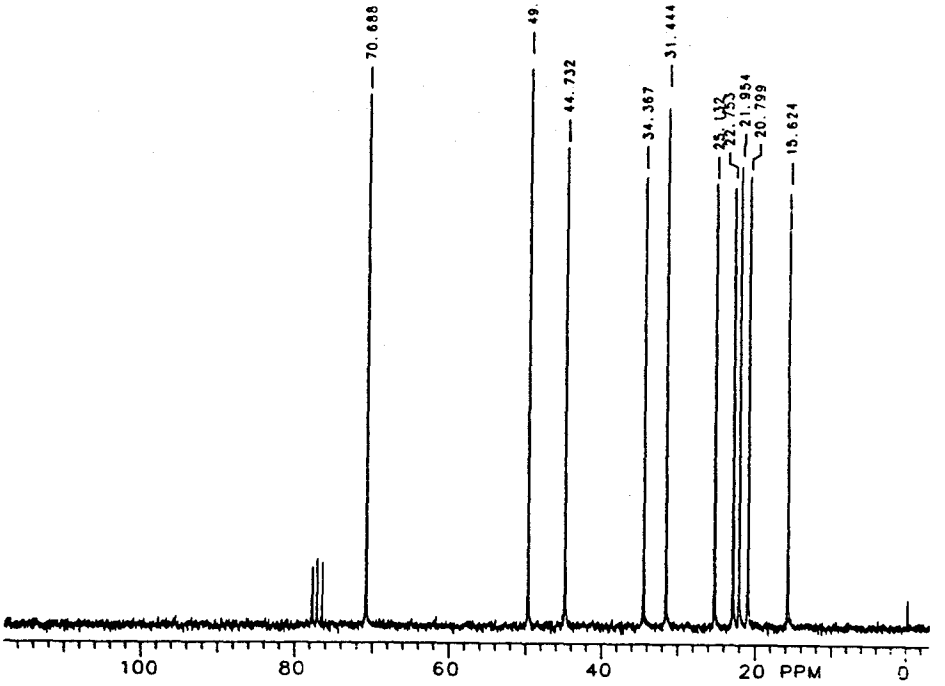


Figure 1: $^{13}C\{^{1}H\}$ spectrum of 57% menthol/$CDCl_3$ at 50.3 MHz on the XL200 using standard parameters and 64 acquisitions.

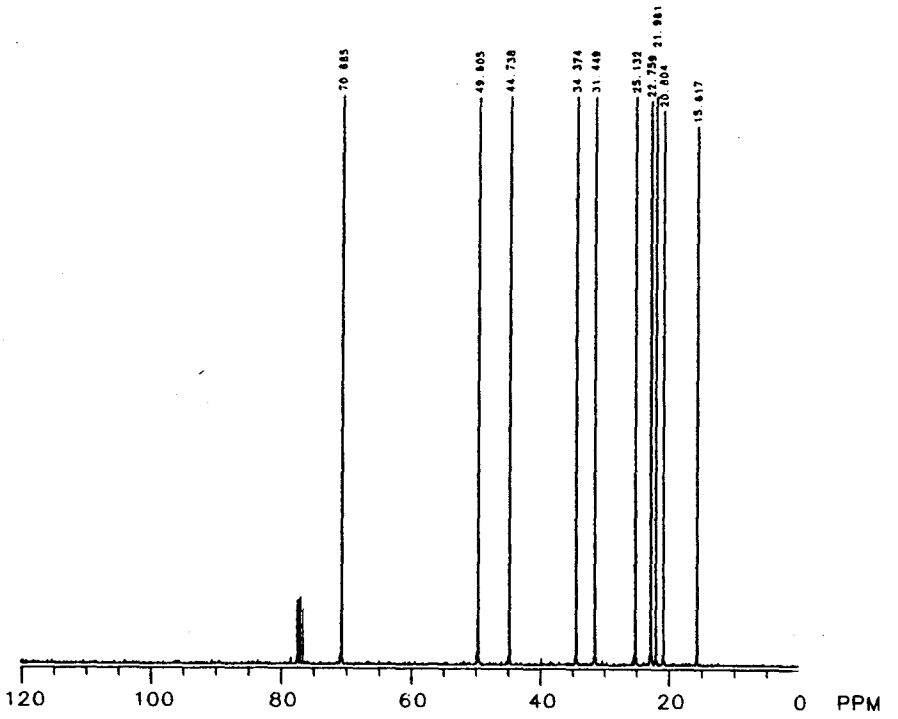


Figure 2: $^{13}C\{^{1}H\}$ spectrum of 57% menthol/$CDCl_3$ at 75.6 MHz on the QE300 using standard parameters and 64 acquisitions.

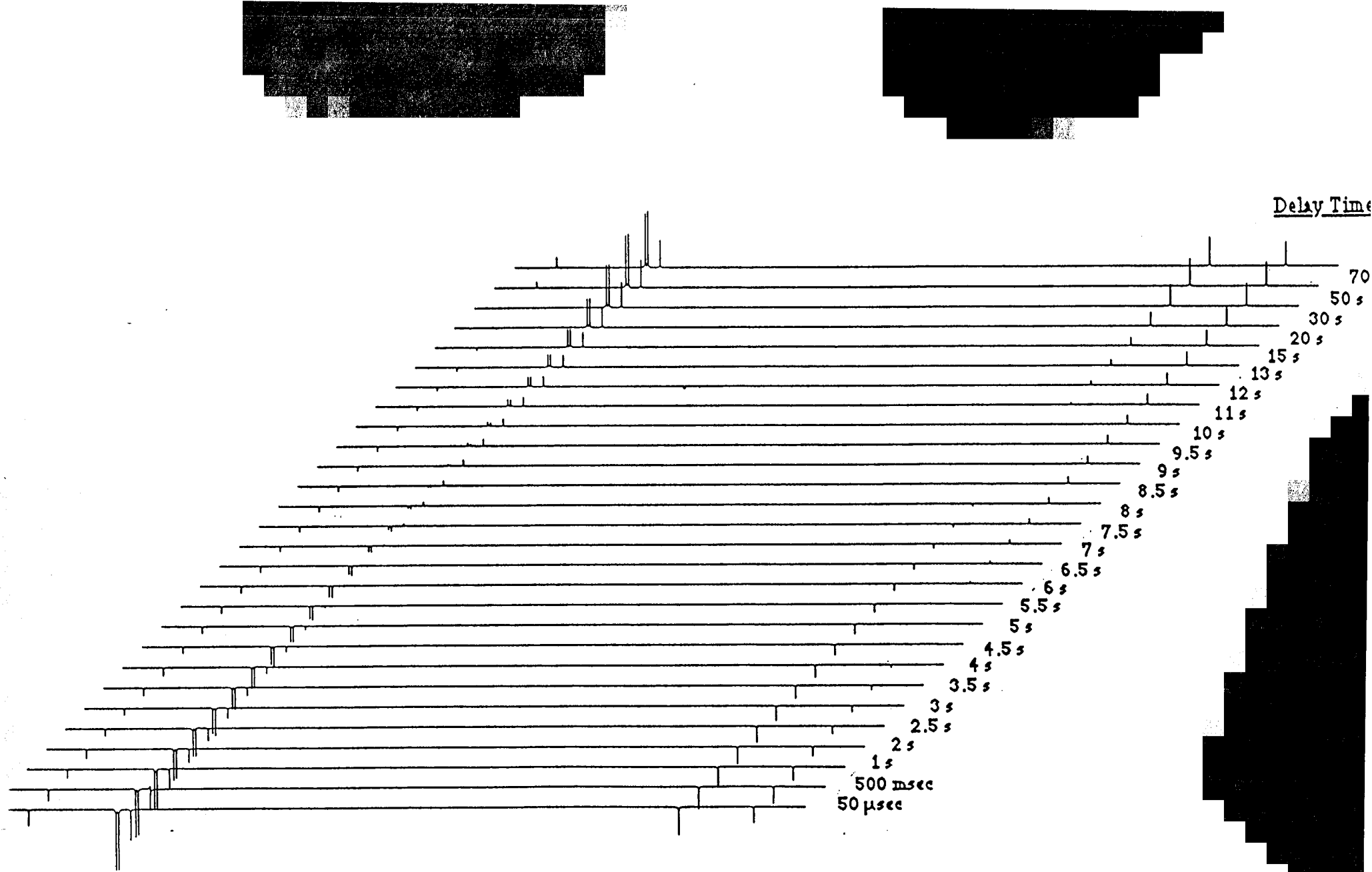


Figure 5: $^{13}C\{^{1}H\}$ study of 90% ethylbenzene/$CDCl_3$ by the inversion recovery method. The delay time which results in an intensity of zero for a particular signal is called that signal's "null time." The T1 can be estimated by dividing the null time by 0.69(ln2).

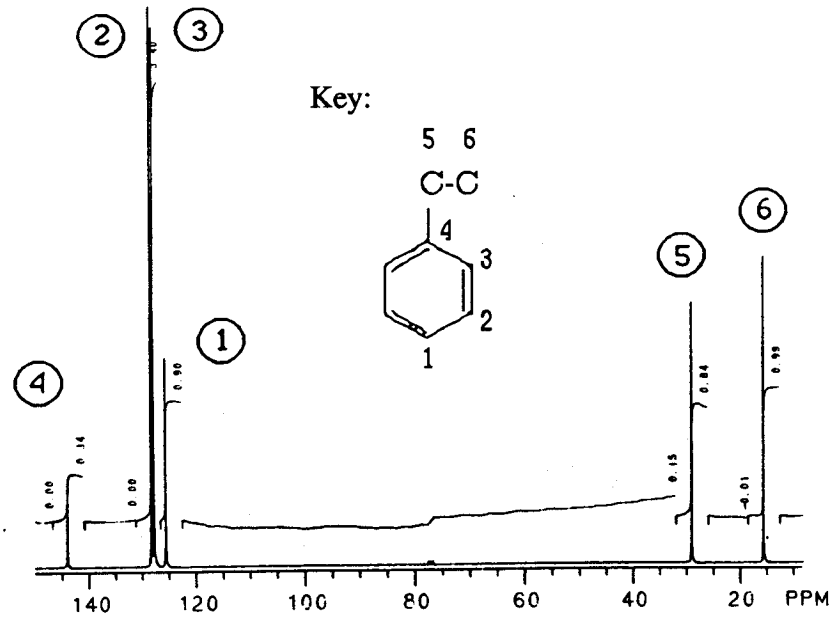


Figure 7a: $^{13}C\{^{1}H\}$ spectrum of 90% ethylbenzene/$CDCl_3$ at 75.6 MHz, decoupler on continuously, 4 acquisitions, **recycle delay of 1.8 seconds**, using a 90 degree flip angle.

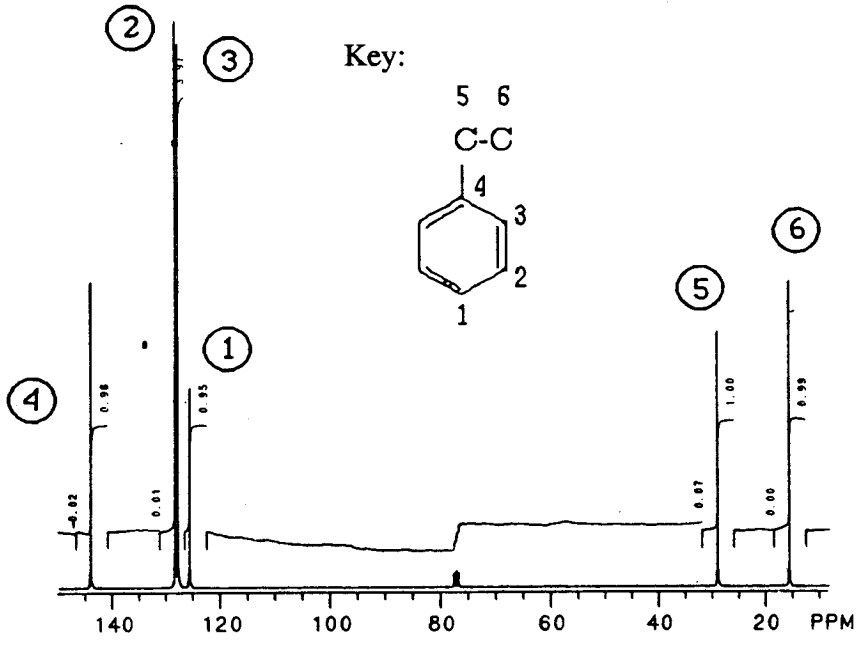


Figure 7b: $^{13}C\{^{1}H\}$ spectrum of 90% ethylbenzene/$CDCl_3$ at 75.6 MHz, decoupler gated <u>on</u> only during the acquisition time, 4 acquisitions, **recycle delay of 5xT1 (longest)**, using a 90 degree flip angle.

Table 1

Spectrum	ICH_3(6)	I(5)	%er	I(4)	%er	I(3)+I(2)	%er	I(1)	%er
Figure 7a	1.00	0.85	15	0.34	66	3.43	14.2	0.91	9
Figure 7b	1.00	1.01	1	0.97	3	4.01	0.25	0.96	4

IV. NMR Basics Test
Level Two Checkout
XL200/QE300

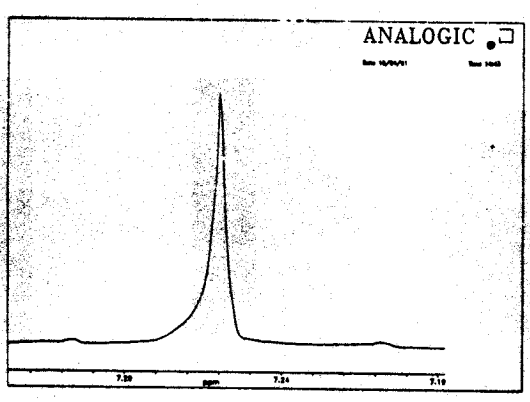
1. Diagram and label the three parts of the 1PULSE FTNMR experiment.

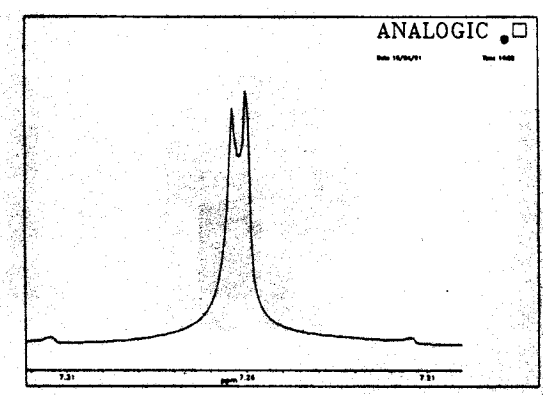
2. What is the result when you apply the FT to an FID (time domain signal)?

3. What is the relationship between numbers of points, spectral width, acquisition time, and 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? Label the shim probably responsible for the distortions below.





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

7. What is the equation for determining signal-to-noise (S/N)?

8. Given that after 100 scans (5 minutes) the S/N for a sample is 10:1 on the XL200, and 35:1 on the QE300, how long will it take to achieve a S/N of 350:1 on each instrument?

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

10. When would you use a homodecoupling experiment?

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