Software/Plotting Practice for Varian/Agilent NMR Spectrometers Operating with VnmrJ2.2D/3.2/4.2A

This handout contains instructions on the processing and plotting of NMR data acquired from a Varian/Agilent NMR spectrometer using the software package VnmrJ2.2D/3.2/4.2A. Older Varian spectrometers used VNMR6.1c and will not be covered in this handout. The purpose of this exercise is to acquaint you with the system as well as some of the most basic commands for data processing and plotting, and to prepare you for the actual training on a spectrometer. You should practice as many times as is necessary until a basic understanding of the new system is acquired and an acceptable proficiency in data processing and plotting is achieved.

NOTE: Even if you plan to do the majority of your data processing off-line using second party processing programs like the ACD NMR program or MNova, you still need a certain proficiency using the Varian/Agilent software for processing in order to check your data.

This handout will cover the following areas:

1. Retrieving a data file.
2. Saving and deleting a data file.
3. Data processing, *e.g.*, weighted Fourier transformation, phasing, referencing, *etc*.
4. Integration of NMR signals.
5. Measuring Signal-to-Noise (S/N) and line-width-at-half-height ($LW_{1/2}$).
6. Displaying and plotting spectra.

Before Getting Started:

According to the instructions, you should have taken the BASICS test before starting with this handout. If you haven't done so, I suggest you do it now. During the course of going through this handout, several questions are asked and they are addressed in the Basics handout. I do expect you to pay attention and answer them. In order to facilitate that process, I reproduce the relevant sections and their questions, below.

Compare the S/N values with *lb*=0, *lb*=0.3, and *lb*=1. What is the advantage/disadvantage of using lb? Compare the *pll* printout with the *ppf* printout. Why would you prefer one to the other? Or do you think it matters?

Why is the integral in the phenyl region ~4 instead of ~5?

How can you tell if your spectrum is "well-phased"?

Part IV. ^{1}H Lineshape, 1.0% $CHCl_3$ in $CDCl_3$ page 12

Note the linewidth and the digital resolution. Why do they differ from each other?
Note the linewidth and the digital resolution. Did they change? Why or why not?
What conclusions can you draw with respect to zero filling and linewidth, line broadening and linewidth, and digital resolution and linewidth?
What value should you expect to obtain for the $LW_{1/2}$ of the $CHCl_3$ peak in this sample when you practice on the spectrometer?

Part V. $^{13}C\{^{1}H\}$ Spectrum of Menthone in $CDCl_3$ page 13

What do you think would happen if these spectra were re-measured using *d1*=5?
If after 2 hours of acquisition, some of the signals in your ^{13}C spectrum are just barely visible, how long would it take to double the S/N so that these signals are more creditable?

Explanation of Types of Commands Found in this Handout:

1. The VNMR software and the Linux operating system are both case sensitive. This means that the computer distinguishes whether the letters are entered in upper case (*i.e.* CAPITALS) or lower case. The user must be careful to type the correct case for each letter in a command.

   Example: **jexp1** is not the same as **JEXP1**

2. Some commands are line commands and are typed in by the user followed by hitting a return (or the Enter key) (signified by <rtn>).

   Example: **wft** <rtn>

3. Some commands are executed by clicking a mouse button with its pointer on a icon found on the screen. The execution of these commands is indicated by a two-letter designation (LC {left click}, RC {right click}, or CC {center click}).

   Example: LC **Main Menu**
   This means to click the left mouse button with its pointer on the button that says "Main Menu".

4. Some commands are executed by using the mouse itself. These commands are indicated by a two-letter designation (LC, RC, or CC) and a description of what the user should do in parentheses.

   Example: LC (at 6 ppm)
   This means to click the left mouse button with the mouse cursor positioned at 6ppm.

5. Parameters are entered by typing the parameter name followed by an equal sign, then the value, and finally hitting return.

   Example: **nt=16** <rtn>

Commands Used in this Handout

Command	Description	Typed Example
aph	Automatic phasing	f full *aph*
cdc	Clear previous drift correction (use with dc)	*cdc* dc
cr=#	Cursor position	*cr?* or *cr=10p*
cz	Clear integral resets	*cz*
da	Display arrayed parameters	*da*
dc	Apply drift correction to get horizontal baseline	*dc*
dg	Display experiment parameters	*dg*
dg1	Display 1st group expt. Parameters	*dg1*
dgs	Display special expt. Parameters	*dgs*
dll	Display peak line list w/ ppm and intensity values	*dll*
dpf	Display peak frequency list on spectrum	*dpf*
dpir	Display peak integral regions under spectrum	*dpir*
dres	Display resolution and $LW_{1/2}$ for cursor position	*dres*
ds	Display spectrum	*ds*
dsn	Display signal-to-noise of largest peak vs. selected noise	*dsn*
dssa	Display stacked spectra automatically (stacked vertically)	*dssa*
dssh	Display stacked spectra horizontally	*dssh*
exit	Exit the VNMR program (must be done before logging off)	*exit*
f	Display full spectrum for given chart width	*f*
fn=#	Fourier number used for zero-filling (improves digital res)	*fn=2*np wft*
ft	Perform a Fourier transform without weighting function	*ft*
full	Resize visible spectrum to fit window	f *full*

Command	Description	Typed Example
isadj	Automatic adjustment of integral trace height	*isadj*
lb=#	Set line broadening factor for exponential multiplication	*lb=0.3* wft
nl	Move cursor to nearest line	*nl*
page	Send all plotting selections to the printer	pl pscale pir *page*
pap	Plot all parameters	pl pscale *pap* page
pir	Plot integral region values	pl pscale *pir* page
pl	Plot spectrum line	*pl* pscale page
pltext	Plot text to spectrum	pl pscale *pltext* page
pscale	Plot axis scale below spectrum	pl *pscale* page
rl(*#p*)	Reference current cursor position to parenthetical values	*rl(7.24p)*
sp=*#p*	Start point of spectrum display and plot	*sp=0p* wp=10p
svf	Save data file to hard drive	*svf('filename')*
text('….')	Add text to spectrum (use \\ to open new lines of text)	*text('line1\\line2')*
vp=#	Vertical position of spectral trace in mm (used w/ dpir/pir)	*vp=12*
vs=#	Vertical scale in mm	*vs=vs*2*
vsadj	Vertical scale auto-adjustment (largest peak fits screen)	*vsadj*
wft	Weighted Fourier transform	*wft*
wp=*#p*	Width of spectrum display and plot	sp=0p *wp=10p*

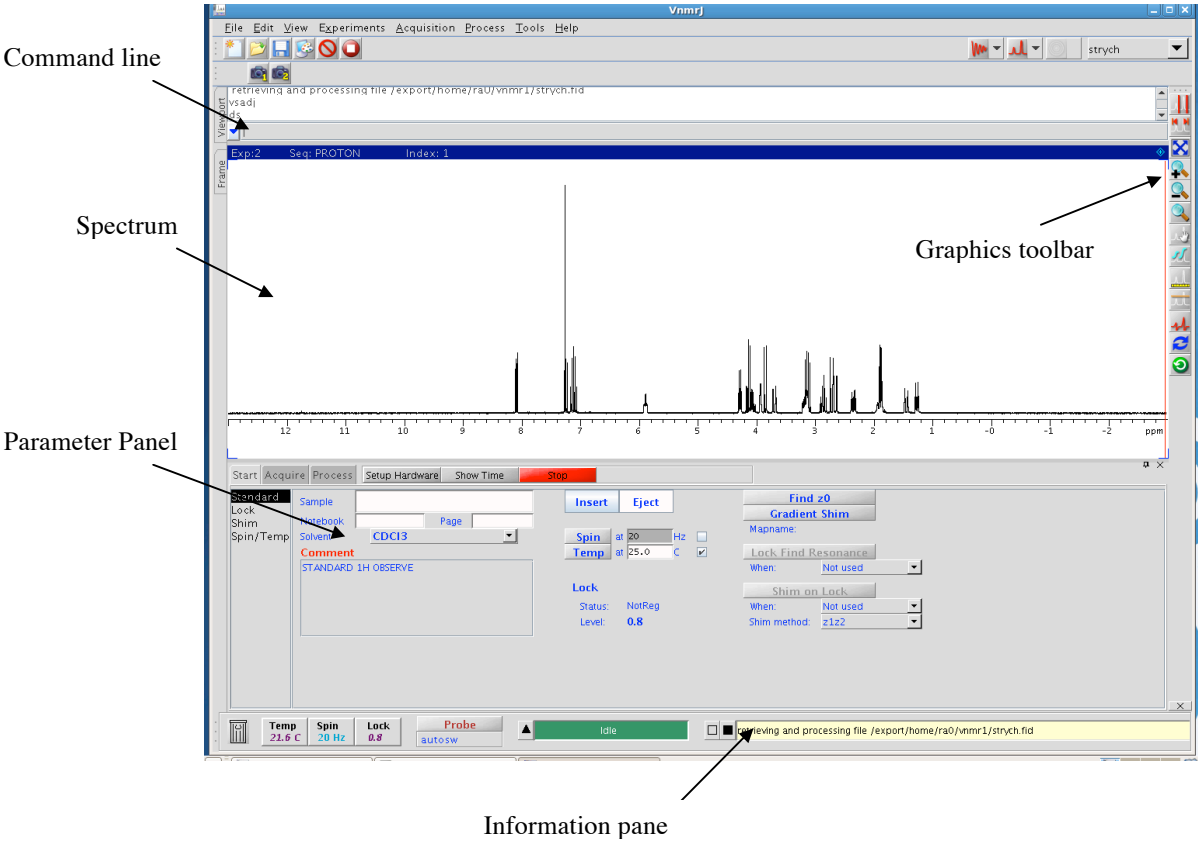
Icon Guide *(icons for VnmrJ4.2A have a blue background)*		
Icon	**Name**	**Action**
	Full Spectrum	Displays the full spectral region.
	Magnifying Glass	Expands a region when user clicks and drags to highlight region.
	Pan & Stretch	Left click allows user to move to new spectral region. Right click and hold will either expand (drag up) or contract (drag down) spectrum.
	One Cursor	Selects single cursor mode.
	Two Cursors	Selects double cursor mode.
	Define Integral Regions	Enters interactive Integral mode and allows user to define integral reset points.
	Show Integral Regions	Displays Defined integral regions.
	Show Full Integral	Displays full integrals including regions that have no defined integral.
	Hide Integral	Hides integral traces from display.
	Show/Hide Threshold	Enters/Leaves interactive peak selection mode.
	Show/Hide Axis	Displays/Hides scale.
	Phase Mode	Enters manual phasing mode.
	Return	Returns to previous menu.
	1D spectrum	Displays interactive 1D spectrum and 1D menu.
	Save As…	Opens interactive save feature.
	Open…	Opens user NMR data folder.
	Cancel Command	Cancels current command.
	Stop Acquisition	Stops an experiment that is running.

To start the practice, log onto a Datastation (subbasement B-8, South wall, two computers):

1. At the computer keyboard type your Plotting Practice Username (obtain this username from the staff) and hit Return <rtn>. If it is already logged in, you'll only need to enter the password.

2. To start *VnmrJ*, double click (LC) on the icon labeled VnmrJ.

3. The VnmrJ interface looks like the following:



Part I. ^{1}H Sensitivity, 0.1% Ethylbenzene in $CDCl_3$

Load the SNH1.fid Directory

--Previously acquired data are stored as directories containing the FID, log, text, acquisition and processing information. In order to process them, they must be loaded.

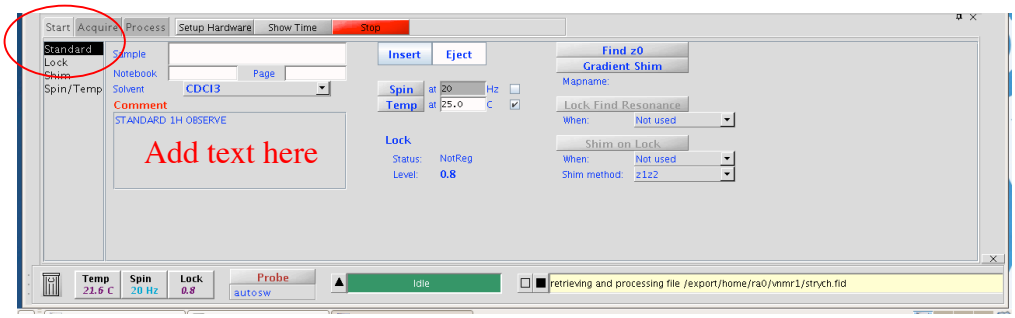
LC 	Open data directory
Double LC **SNH1.fid**	load the SNH1.fid directory and do preliminary processing

IMPORTANT: You should stay in this directory for the duration of the practice. Do

not delete or save over the data in this directory!

Display Parameter Panel and Command Line

LC *View, Parameter Panel* — Display Parameter Panel
LC *View, Command Line* — Display Command Line
LC *Start* Tab in Parameter Panel and select *Standard* (see below):



Enter Text, Transform, Display, and Phase the Spectrum

In the Comment Field, enter *the date*, *your name*, and ***SNH1***. The Sample, Notebook, and Page fields are optional areas for entering more information regarding your data.

wft <rtn> — weighted Fourier transformation
LC  — display full spectrum
aph <rtn> — autophase

Hide/Show Parameter Panel

LC  (in upper right of parameter panel). This is the thumbtack icon.

Place the pointer (cursor) over the Parameter panel tab at the bottom right of the spectrum and LC the  to pin the panel in place.

Reference the Spectrum

NOTE: You would typically reference the spectrum to the residual solvent peak (in this case, the $CHCl_3$ peak). This is not ideal for the practice sample because the solvent peak overlaps with compound peaks. We will, therefore, reference the TMS (tetramethylsilane) to Ø ppm.
You should always expand around the peak to be referenced and make sure that the red cursor is on the proper peak before typing the reference command, rl(##p).

If no there is scale on spectrum, LC  .
LC  — enter expansion mode

LC on spectrum at 0.1ppm and hold the button to highlight to –0.1ppm then release mouse button to expand.
vsadj <rtn> (or CC on top of peak at the desired height) adjust vertical scale

LC  If not available on graphics toolbar, LC the down arrow to the right of  at top right and choose One Cursor option.

LC (at the center of the highest peak) this places the red cursor on the TMS signal
nl <rtn> select nearest line
rl(0p) <rtn> reference the selected line to 0 ppm

Measure S/N and Store Value in Register

You are going to determine the Signal-to-Noise (S/N) of the smallest peak of the compound, which is at 2.66 ppm. Good integration requires high S/N (at least 100).

LC  display full spectrum (same as typing **f** <rtn>)

LC  enter expansion mode

LC on spectrum at 6 ppm and hold the button to highlight to 2 ppm then release mouse button to expand.

vsadj <rtn> (or CC on top of peak at the desired height) adjust vertical scale

LC 

LC (at 5.5 ppm)
RC (at 4.0 ppm)
There should be two red cursors on the spectrum: one at 5.5 ppm and the other at 4 ppm)

dsn <rtn> (The value should be ~142.) measure signal-to-noise, with the quartet as the signal and 5.5 – 4.0 ppm as the noise region
The S/N is _____ note the signal-to-noise ratio

lb? <rtn> Display line broadening value
lb value is _____

Plot Spectrum with Scale and Parameters

pl <rtn> plot spectrum
pscale <rtn> plot scale
pap <rtn> plot parameters
page <rtn> submit above plotter commands to the plotter and change paper after the plot has been completed

NOTE: These commands can be strung together into a single command (e.g. ***pl pscale pap page***).

Reprocess the data with a different line broadening value

The original value was 1. Now, we'll see the results of changing this value. First, we'll perform the Fourier Transform without weighting (ft).

ft <rtn>	Fourier transform without applying weighting function. This is equivalent to wft with lb=0 (default setting for ^{1}H)
dsn <rtn>	display S/N

S/N value is _____

Reprocess the data with yet a different line broadening value

lb=0.3 <rtn>	change value of line broadening
wft <rtn>	weighted Fourier Transform
dsn <rtn>	measure signal-to-noise, with the quartet as the signal and 5.5 – 4.0 ppm as the noise region

S/N value is _____

Reprocess the data with yet a different line broadening value

lb=10 <rtn>	change value of line broadening
wft <rtn>	weighted Fourier Transform
dsn <rtn>	measure signal-to-noise, with the quartet as the signal and 5.5 – 4.0 ppm as the noise region

S/N value is _____

***Look** at the peak at 2.66 ppm. Is it still a quartet? Would you use this lb value?*

QUESTION: *Compare the S/N values and peak widths with lb=1, lb=10, lb=0.3, as well as using ft only. Of the lb values used, which would be preferred and why?*

Set Threshold for Peak Picking and Display Frequencies on Screen

ds <rtn>	display interactive spectrum
lb=0.3 wft vsadj <rtn>	reduce lb to 0.3 Hz, transform data, and auto-adjust the vertical scale.
LC 	select threshold menu
LC	(drag the horizontal orange cursor up or down to set the threshold level, excluding the small peak at 3.7 ppm) – everything above the orange line will be picked.
dpf <rtn>	display peak frequencies above the spectrum

Note that the spectrum is no longer interactive. To make interactive, LC  ,  or

type **ds** <rtn>.

Plot Spectrum with Parameters and Peak Frequencies

pl *ppf* pscale pap page <rtn> plot spectrum *with peak picking* in ppm

Plot Peak Listing in Hz and ppm

pll page <rtn> plot peak frequencies *in both Hz and ppm*

Question: *Compare the pll printout with the ppf printout. Why would you prefer one to the other? Or do you think it matters?*

Plot Peak Listing in Hz and ppm

ds <rtn> display interactive spectrum

Place pointer over the tallest peak at the top of the spectral window and MM (middle mouse click). This will bring the spectrum to the pointer. Place pointer underneath the scale and MM. This halves the scale for each click.

Part II. ^{1}H Integration, 0.1% Ethylbenzene in $CDCl_3$

Load the **INTH1.fid** Directory

LC  Open data directory

Double LC **INTH1.fid** load the INTH1.fid directory and do preliminary processing

Enter Text, Transform, Display, and Phase Spectrum

In the Comment Field, enter *the date*, *your name*, and ***INTH1***. The Sample, Notebook, and Page fields are optional areas for entering more information regarding your data.

wft <rtn> weighted Fourier transformation

LC  display full spectrum

aph <rtn> autophase

Reference the Spectrum (setting the TMS peak to 0 ppm)

NOTE: *This is exactly the same type of procedure used in Part I.*

If no there is scale on spectrum, LC  .

LC  enter expansion mode

LC on spectrum at 0.1ppm and hold the button to highlight to –0.1ppm then release mouse button to expand.
vsadj <rtn> (or CC on top of peak at the desired height) adjust vertical scale

LC  If not available on graphics toolbar, LC the down arrow to the right of  at top right and choose One Cursor option.

LC (at the center of the highest peak) this places the red cursor on the TMS signal
nl <rtn> select nearest line
rl(0p) <rtn> reference the selected line to 0 ppm

Integration

NOTE: *When you are in the Integral mode, the middle mouse button (MM) controls the size of the integrals and not the spectrum. To control the spectrum size, you have several options: (1) type* ***vsadj*** *<rtn> to bring the largest peak to the top of the screen; (2) type vs=vs*2 ds <rtn> to double the scale; (3) and lastly, LC the arrow to the right of*  *, choose Integrals, Hide Integrals. The MM will now control the spectrum. You will need to Choose Partial Integrals under the same menu to return to the Integral mode.*

LC  enter integration routine with integral blanking

f <rtn> or LC  display full spectrum
cz <rtn> clear previous integral reset points, if any
cdc <rtn> cancel previous drift correction, if any
dc <rtn> apply drift correction

NOTE: When setting integrals, try to include only the peaks of interest and to be consistent in the amount of baseline cut on either side of your peaks

Expand *around the region of interest*; I recommend that you start with the phenyl region. Select the areas to be cut by using:

LC  sets mode for integral selection

LC on the **left and right** sides of the signal(s) to set integral zero points.

Repeat this process for the rest of the signals you want to integrate in this area; then

LC  . Click and drag to pan to the next region or LC  and  to zoom the next region. *Use* ***vsadj*** *<rtn> to resize the spectrum to help with setting the integrals.*

NOTE*: Every time you switch to another expansion, you will need to LC*  *. If you*

make a mistake, you can use a Right mouse Click (RC) at the last position that will undo the last reset point.

wp=10.5p <rtn> set width of plot
sp=-0.5p <rtn> set start of plot
vsadj <rtn> adjust vertical scale
isadj <rtn> (or CC on top of an integral at desired height) adjust integral vertical scale
ds <rtn> display spectrum

LC 
Place *the cursor on the peak or integral cut you want to be normalized by clicking on that peak. The vertical red line should cross the green integral trace. Choose the quartet.*

LC *View, Parameter Panel*

LC *Process tab* in Parameter Panel

LC *Integration* (on left of Parameter Panel)

Change the Integral Area field to desired value (In this case, ***2****)*

LC *Set Integral Value*

dpir <rtn> display the integral values below the spectrum. Close parameter panel if integral is obscured
ds <rtn> display spectrum

Question: This is a spectrum of ethylbenzene. It should have integrals in the ratio of 5:2:3. *Why is the integral in the phenyl region ~4 instead of ~5? (Hint: Type* ***d1?*** *<rtn> and note the value.*

Plot Spectrum with ***Integrals*** and ***Integral values***

pl *pir* pscale pap page <rtn> plot with *integrals* on the spectrum and integral values under the spectrum

Reusing Old Commands

LC arrow to the left of the command line
Scroll to the top of the recent command list and click on the pl pir… command you just used. Change ***pir*** *to* ***ppf*** *and Hit <ENTER>.*

Turn off Integral Display

ds <rtn> or *LC* 

vp=0 <rtn> set vertical position of spectrum back to zero

Part IIa. Baseline Correction of a ^{1}H Presaturation Experiment

Baseline correction is used to correct for a distorted baseline due to instrumental artifacts and background signals. A flat baseline will improve the accuracy of integration. In order to perform baseline correction, we'll need to set the integrals. Every peak in a spectrum will need to be integrated even if it is not a desired signal.

Load **yellow_soda.fid** Directory and add text

LC 	Open data directory
Double LC **yellow_soda.fid**	load the yellow_soda.fid directory and do preliminary processing

In the Comment Field, enter *the date*, *your name*, and ***baseline correction***. The Sample, Notebook, and Page fields are optional areas for entering more information regarding your data.

Manually Phase Spectrum

You'll notice that the autophasing is not acceptable. We will, therefore, manually phase the spectrum.

ai vs=1000 <rtn>	sets absolute intensity mode and vertical scale to 1000 in order to see the phasing issue

Now, we'll do manual phasing:

Manual Phasing

LC 	enter the interactive phasing mode

LC (click on a signal toward the right side of the spectrum about halfway vertically up the screen and adjust the phase by moving the mouse vertically while holding down the left button for coarse adjustment, or the right button for fine adjustment, of the zero-order or frequency-independent phase parameter *rp*)

LC (click on a signal toward the left side of the spectrum and adjust the phase as above to change the first order or frequency dependent phase parameter *lp*)

LC 	exit the interactive phasing mode

NOTE: if you can't seem to phase the spectrum manually, reset both zero order and first order phases to zero by typing "**lp=Ø** <rtn>" and "**rp=Ø** <rtn>", and try again.

Set vertical scale and perform drift correction

vs=6000 <rtn>	sets a large vertical scale to see baseline

	issues
cdc dc <rtn>	clears previous drift correction and applies new correction. This sets the ends of the spectrum to a straight line

Note that the middle of the spectrum bows up. We'll fix this with baseline correction.

Integration for baseline correction

Full spline baseline correction requires integration of all peaks. This algorithm will force all non-integrated areas to have a zero integral. This will result in large distortions if peaks are not integrated.

LC 	enter integration routine with integral blanking
f <rtn>	or LC  display full spectrum
cz <rtn>	clear previous integral reset points, if any

Expand *around the region of interest*; I recommend that you start with the phenyl region. Select the areas to be cut by using:

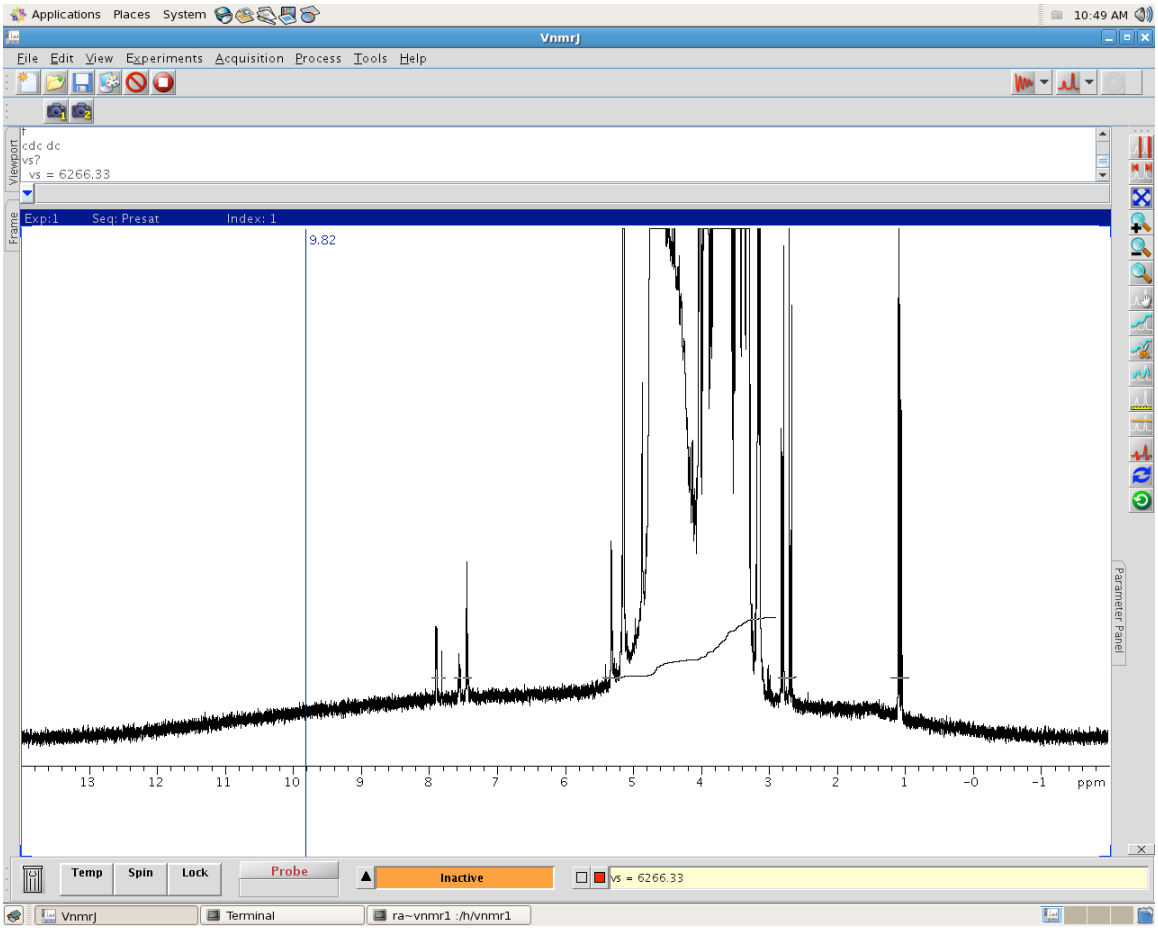
LC 	sets mode for integral selection

LC on the **left and right** sides of the signal(s) to set integral zero points.

LC  . Click and drag to pan to the next region or LC  and  to zoom the next region. *Use* ***vsadj*** *<rtn> to resize the spectrum to help with setting the integrals.*

NOTE*: Every time you switch to another expansion, you will need to LC*  *. If you make a mistake, you can use a Right mouse Click (RC) at the last position that will undo the last reset point.*

Repeat this process for the rest of the signals in this spectrum. You can set one integral region from 5.5 ppm to 3ppm. It should look like the following;



Apply baseline correction

bc <rtn> — applies baseline correction. A wft will undo bc

Plot Spectrum

pl pscale page <rtn> — print 'oversized' spectrum

Part III. ^{1}H Homodecoupling, 0.1% Ethylbenzene in $CDCl_3$

Load **DECH1.fid** Directory

LC  — Open data directory

Double LC **DECH1.fid** — load the DECH1.fid directory and do preliminary processing

In the Comment Field, enter *the date*, *your name*, and ***DECH1***. The Sample, Notebook, and Page fields are optional areas for entering more information regarding your data.

Auotmatic processing gives three stacked spectra. You'll notice, however, that phasing is

poor. This is particularly evident for the multiplet at 7.2 ppm. Let's take a closer look.

LC 
vsadj <rtn>

All peaks should be purely absorptive (i.e. above baseline). The artifact at 5.15 ppm (due to decoupling) is interfering with the automatic phasing. We'll need to phase these spectra manually.

wft <rtn>	weighted Fourier transformation
ds(1) <rtn>	display spectrum 1 (the control spectrum)
f full aph <rtn>	display full spectrum to full screen and autophase

Question: *How can you tell if your spectrum is "well-phased"?*

Manual Phasing

LC 	enter the interactive phasing mode

LC (click on a signal toward the right side of the spectrum about halfway vertically up the screen and adjust the phase by moving the mouse vertically while holding down the left button for coarse adjustment, or the right button for fine adjustment, of the zero-order or frequency-independent phase parameter *rp*)

LC (click on a signal toward the left side of the spectrum and adjust the phase as above to change the first order or frequency dependent phase parameter *lp*)

LC 	exit the interactive phasing mode

NOTE: if you can't seem to phase the spectrum manually, reset both zero order and first order phases to zero by typing "**lp=Ø** <rtn>" and "**rp=Ø** <rtn>", and try again.

Set Plot Limits

wp=2p <rtn>	set width of plot
sp=1p <rtn>	set start of plot

Display Stacked Spectra Vertically

vsadj(80/4) <rtn>	adjust vertical scale to 1/4 of total **vs**
dssa <rtn> or LC 	display stacked spectra vertically

Stacked Plot of Spectra as Displayed

pl('all') pscale page <rtn>	stacked plot of spectrum 1 to 3 with scale

Part IV. ^{1}H Lineshape, 1.0% $CHCl_3$ in $CDCl_3$

Load **LSH1.fid** Directory

LC  Open data directory

Double LC **LSH1.fid** load the LSH1.fid directory and do preliminary processing

Enter Text, Transform, and Phase the Spectra

In the Comment Field, enter *the date*, *your name*, and ***LSH1***. The Sample, Notebook, and Page fields are optional areas for entering more information regarding your data.

wft <rtn> weighted Fourier transformation
f <rtn> display full spectrum to a full screen
aph <rtn> autophase (manually phase it if necessary)
dscale <rtn> display scale in Hz (because *axis = 'h'*)

Reference the $CHCl_3$ signal to 0 Hz

LC  and highlight around the left peak

LC 

LC (at the center of the peak)
nl <rtn> select nearest line
rl(0) <rtn> reference the selected line to 0 Hz

Determine $LW_{1/2}$ and Note Value

dres <rtn> measure linewidth at half-height for selected signal

Linewidth is _____
Digital Resolution is _____

Question: *Note the linewidth and the digital resolution. Why do they differ from each other?*

Reprocess the Data with Zero-filling

Zero-filling is simply adding data points with zero intensity to the end of the FID. This will add data points to your FID without adding additional noise and thereby improve digital resolution. It is important to note, however, that zero-filling does not improve true resolution; it only improves the apparent resolution.

fn=2*np <rtn> set zero-filling to twice number of points
wft <rtn> weighted Fourier transformation

LC (at the center of the peak)
nl dres <rtn> select nearest line and display $LW_{1/2}$

Linewidth is _____
Digital Resolution is _____

Question: *Note the linewidth and the digital resolution. Did they change? Why or why not?*

Plot Spectrum and Print Parameter groups

wp=50 <rtn> set width of plot to 50 Hz
sp=-25 <rtn> set start of plot to -25 Hz. Note the minus sign
vsadj <rtn> adjust vertical scale
pl pscale pap page <rtn> plot spectrum with scale and parameters

Reprocess the Data with lb=1

lb=1 <rtn> set line broadening to 1 Hz
wft <rtn> weighted Fourier transformation
LC (at the center of the peak)
nl dres <rtn> select nearest line and display $LW_{1/2}$

Linewidth is _____
Digital Resolution is _____

Question: *What conclusions do you draw with respect to zero-filling and linewidth, line broadening and linewidth, and digital resolution and linewidth?*

Part V. $^{13}C\{^{1}H\}$ Spectrum of Menthone in $CDCl_3$

Load **MEN.fid** Directory

LC  Open data directory
Double LC **MEN.fid** load the MEN.fid directory and do preliminary processing

Enter Text, Transform, Display, and Phase Spectrum

In the Comment Field, enter *the date*, *your name*, and ***MEN***. The Sample, Notebook, and Page fields are optional areas for entering more information regarding your data.

ds(1) <rtn> display spectrum 1 (nt=4)
f aph <rtn> display full spectrum to a full screen and autophase
ds(4) <rtn> display spectrum 4 (nt= 256)

Look *at the resonance around 210 ppm. Is this resonance properly phased? No, it is*

not. Automatic phasing on low S/N samples (spectrum 1 with nt=4) is not effective. If you must phase such a spectrum, manual phasing is necessary. In the current case, the higher S/N sample can be used for phasing.

f aph <rtn> display full spectrum and automatic phase

Now the 210 ppm peak looks more reasonable. It still could use manual phasing. You can try to phase it if you like. Refer to bottom box on page 8 for manual phasing.

***Expand** around the 77 ppm region and place the cursor on top of the center peak of the small triplet.*

nl rl(77p) <rtn> reference the carbon triplet of the solvent to 77.0 ppm.

***Why is the ^{13}C peak of $CDCl_3$ a triplet?** Answer: In ^{13}C NMR, the solvent peak is split due to deuterium (^{2}H) coupling. Splitting is calculated by the equation, splitting=2nI+1, where n is the number of attached nuclei and I is the nuclear spin quantum number. Unlike, ^{1}H and ^{13}C nuclei that are spin $\frac{1}{2}$ nuclei (nuclear spin quantum number, I, is 1/2), ^{2}H has a spin 1 nucleus (I = 1). Therefore, the carbon of a solvent like $CDCl_3$ will be a triplet (splitting=2(1)(1)+1=3).*

Select Noise Region and Measure S/N for Spectrum 1

ds(1) <rtn> display spectrum 1 (nt=4)
f <rtn> display full spectrum
LC (at 120 ppm)
RC (at 80 ppm)
dsn <rtn> (should be ~15) measure S/N ratio using the largest peak in display as the signal and 120 - 80 ppm as the noise region

S/N = _____

Measure S/N for Spectrum 2

ds(2) <rtn> display spectrum 2 (nt=16)

LC  brings up last cursor positions
dsn <rtn> measure S/N (120 - 80 ppm)

S/N = _____

Measure S/N for Spectrum 3

ds(3) <rtn> display spectrum 3 (nt=64)

LC  brings up last cursor positions
dsn <rtn> measure S/N (120 - 80 ppm)

S/N = _____

Measure S/N for Spectrum 4

ds(4) <rtn> display spectrum 4 (nt=256)

LC  brings up last cursor positions
dsn <rtn> measure S/N (120 - 80 ppm)

S/N = _____

Notice that with d1=1, quadrupling the number of transients does not quite double the signal-to-noise ratio of the spectrum for the first two data sets, due to incomplete relaxation of the nucleus between pulses.

Question: *What do you think would happen if these spectra were re-measured using d1=5? Why?*

Question: *If after 2 hours of acquisition, some of the signals in your ^{13}C spectrum are just barely visible, how long would it take to double the S/N so that these signals are more creditable?*

Set Plot Limits

wp=220p <rtn> set width of plot
sp=0p <rtn> set start of plot

Set Threshold for Peak Picking and Display Frequencies on Screen

LC  select threshold menu
LC (drag the horizontal cursor up or down to set the threshold level, excluding the solvent signals) – everything above the orange line will be picked.
dpf <rtn> display peak frequencies above the spectrum

Stacked Plot the Spectra Horizontally

dssh <rtn> display stacked spectra horizontally
pl('all') pap page <rtn> stacked plot all spectra with parameters

Reprocess the Data with lb=1

lb=1 <rtn> set line broadening weighting function
wft <rtn> weighted Fourier transformation
ds(1) <rtn> display spectrum 1
f aph <rtn> display full spectrum and autophase

***Measure** the S/N for these four spectra as above. Then*

Notice that the S/N ratios for these spectra are significantly lower than those processed with *lb=0.5*. ***In general, the best S/N ratio for a spectrum is obtained when lb = $LW_{1/2}$,***

measured on the relevant line after an ft, i. e., the natural linewidth. The ^{13}C linewidths for this sample range from 0.3 to 0.7 Hz.

Part VI. Saving and Deleting a File

Saving a File

svf('*your filename*') <rtn>	save file under name 'your filename'. You should think about strategies for naming files, such as III283H, where III is the notebook number, 283 is the page number, and H indicates a proton spectrum

Alternatively, you can save a file using the following:

LC 	open interactive save feature
Double LC userfiles	select file storage/retrieval menu

In Filename field, remove existing text and input desired filename without spaces.

Deleting a File

Minimize the VnmrJ interface

Double LC process' Home folder on the Desktop

Double LC userfiles folder

RC your data folder and choose Move to Trash

Part VII. Logging off

After you are done, please leave the instrument as you found it.

In the VnmrJ window command line:

exit <rtn> or LC *File, Exit VnmrJ* exit VNMR

IMPORTANT: YOU MUST EXIT VNMR PRIOR TO LOGGING OUT OF YOUR UNIX SESSION.

After VnmrJ closes, click System (at top), Log Out process…

Click Log Out to completely exit the session.

Part VIII. Archiving Data

IMPORTANT NOTE: After all your hard work to synthesize, isolate, and characterize your compounds, it would be terrible to lose that precious data. The NMR staff strives to maintain the integrity of the data stored on the instruments, but we are not an archiving service and cannot guarantee your data on the instruments for more than a week. In this age of hackers and other potential saboteurs, it is very important to archive your data on your personal or group's computer(s) and on disk.

Insert a thumb drive into the USB port. Wait for the thumb drive folder to load. Open the user1d directory and then the userfiles directory. Click and drag your data directory to your thumb drive.

- When you can complete this Plotting Practice with minimal trouble, please bring a set of the printed spectra to the NMR staff to schedule spectrometer training.

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