

PINMRF

Varian 300 MHz NMR Spectrometers Training Guide for Basic 1D NMR Spectroscopy

INCLUDING:

Inova-300-1 w/ 5mm 4-nucleus probe – 365 WTHR

Inova-300-2 w/ 5mm 4-nucleus probe – 4100 BRWN

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Basic Spectrometer Operation Guidelines – 1D Spectra

Varian 300 MHz NMR Spectrometers

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Please note the following generalities

- Under no circumstances lean against or otherwise move the magnet.
- The Varian Vnmr software enables the user to easily access and change functions, options, and their properties. If you are not sure about an option, button or command contact PINMRF staff for assistance.
- If you encounter problems at any time during locking, shimming, acquiring you should halt the experiment and reload the standard parameters for your experiment.
- In order for shimming to work well, samples should be filtered and prepared in good-quality tubes. Sample volume should be at least 0.7 mL. Do not use more than 0.1% TMS.
- The VNMR software works best with the Common Desktop Environment (CDE) graphics on the Sun computer. Please check when you log in that you are using CDE.

Conventions in this guide

- Buttons are depicted with small caps in bold, e.g. **ABORT ACQ**.
- Commands entered in the command window are described in geneva font, e.g. **h1cdcl** , and are executed by hitting the return key.

Important basic functions

- **ABORT ACQ** button -> aborts/halts acquisition
- **RESIZE** button -> enlarges/reduces spectrum window
- **FLIP** button -> flips the spectrum window over the display window and vice versa
- **jexpX** command -> switches to experiment number x (1, 2, 3, 4, etc.)
- **ds** command -> switches back to interactive display mode

Logging on to the Sun computer and starting VNMR

1. In order to log in enter your login ID followed by the password into the welcome screen.
2. Click the Vnmr icon to start the Vnmr software

Sample change

3. **e** (ejects existing sample)
4. Remove sample from spinner and replace it with your cleaned (use Kimwipes) sample
5. **i** (inserts new sample)

Acquisition of ¹H-NMR Spectra

6. **jexp1** (go to experiment 1)
7. **h1xxxx** (loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmsol, meth; for benzene use xxxx = cdcl)
8. **acqi** (opens acquisition window)
9. click **LOCK** to open lock sub-window, then set and/or check the following:
 - turn on sample spinning (if not on already) by setting the spin value to 20 (Hz.)
 - the sample should be locked - lock should be established automatically
 - decrease lock power in steps of 4 units until lock level is between 30 - 60% and is stable
 - if sample is not locked, increase lock power by 16, wait for lock to be established, then decrease lock power in steps of 4 units until lock level is between 30 - 60%
 - do not change the Z0 and lock phase values
10. click **SHIM** to open shim sub-window
11. adjust **Z1C** and **Z2C** (if the lock level increases beyond 100, decrease the lock gain - this can be altered in the current window)
12. click **CLOSE**
13. **nt=XX** (sets number of scans; default is nt=8)
14. **ga** (starts acquisition) - wait for acquisition to finish (beep will sound)
15. **aph** (automatic phasing)

for manual phasing:

- click **PHASE**
- left click on highest peak
- with the left mouse button pressed move cursor up or down in order to adjust the phasing for this peak (NOTE: right mouse button allows for fine phase control)
- then left click on farthest peak and repeat phasing
- interactively phase these two peaks until phasing is completed
- **ds** (switches back to interactive display mode)

16. **vsadj** (automatic adjustment of vertical scale -> highest peak adjusted to maximum of spectrum window)

for manual vertical scale adjustment:

- use the center mouse button, a left mouse click above the baseline causes the spectrum to scale up and a left click below the baseline causes the spectrum to scale down, the distance of the cursor from the baseline while clicking determines the multiplication factor of the in- and decrease

alternative vertical scale adjustment:

- **VS=XXX**, the current value of the vertical scale (vs) is displayed in the lower portion of the spectrum window

17. expand spectrum:

- with left mouse button drag red left spectrum limit to its position (cr on bottom of spectrum = ppm)
- with right mouse button click and drag red right limit to its position (delta = difference in ppm)
- click **EXPAND** (expands spectrum to its set limits)

18. integration:

- NOTE: the button for integration consists of three sequential choices: **PART(ial)** **INTEGRAL**, **FULL INTEGRAL**, **NO INTEGRAL**; the button shows the next mode that can be accessed, not the current integration mode
- click **PART INTEGRAL** (green integral trace appears; see also ‘**Adjustment of Integral Trace**’ at the end of this document)

- **CZ** (clears currently defined integral)
- click **RESETS**
- click the left mouse button closely to the left of the left-most peak
- click the left mouse button closely to the right of the left-most peak
- repeat for each peak or group of peaks across the spectrum (clicking the right mouse button at any time anywhere in the spectrum window causes an undo of the last integral section)
- click **FULL INTEGRAL**
- with the left mouse button position red vertical line on desired integral section
- click **SET INT**
- enter desired value, type return (the display window generates a list of integrals)
- click **NO INTEGRAL**

19. set peak picking threshold (to view peak labels see step 20, below):

- click **TH**
- drag the yellow line to the desired limit
- click **TH**

20. view peak labels and/or spectrum scale:

- the command **dpf** can be used to display the peak labels
- the command **dscale** can be used to display a scale beneath the spectrum
- type **ds** to clear the labels and/or scale and redisplay the spectrum alone

21. **text('xxxxx')** (enters a title to be printed on top of your spectrum)

22. **print**, **printp**, **printi** or **printpi** (i = with integration, p = with peak picking)

Proceed with additional experiments from step 3, or go to '[Finishing up Acquisition of Spectra](#)'

Acquisition of ¹³C-NMR Spectra (note: ¹H-NMR must have been acquired first)

23. **jexp2** (go to experiment 2)

24. **c13xxxx** (loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmso, meth; for benzene use xxxx = cdcl). NOTE: if the lock level drops considerably, re-shim the sample as described for ^1H spectra

25. **nt=xx** (sets number of scans; default is 256)

26. **ga** (starts acquisition) - wait for acquisition to finish (beep will sound)

27. **aph** (automatic phasing, the software does a good job if the signal-to-noise ratio is good)

for manual phasing:

- click **PHASE**
- left click on highest peak
- with the left mouse button pressed move cursor up or down in order to adjust the phasing for this peak
- then left click on farthest peak and repeat phasing
- interactively phase these two peaks until phasing is completed
- **ds** (switches back to interactive display mode)

28. **vsadj** (automatic adjustment of vertical scale -> highest peak adjusted to maximum of spectrum window)

for manual vertical scale adjustment:

- use the center mouse button, a left mouse click above the baseline causes the spectrum to scale up and a left click below the baseline causes the spectrum to scale down, the distance of the cursor from the baseline while clicking determines the multiplication factor of the in- and decrease

alternative vertical scale adjustment:

- **VS=XXX**, the current value of the vertical scale (vs) is displayed in the lower portion of the spectrum window

29. expand spectrum:

- with left mouse button drag red left spectrum limit to its position (cr on bottom of spectrum = ppm)
- with right mouse button click and drag red right limit to its position (delta = difference in ppm)
- click **EXPAND** (expands spectrum to its set limits)

30. set peak picking threshold:

- click **TH**
- drag the yellow line to the desired limit
- click **TH**
- the command `dpf` can be used to display the peak labels

31. view peak labels and/or spectrum scale:

- the command `dpf` can be used to display the peak labels
- the command `dscale` can be used to display a scale beneath the spectrum
- type `ds` to clear the labels and/or scale and redisplay the spectrum alone

32. `text('xxxxx')` (enters a title to be printed on top of your spectrum)

33. `print` or `printp` (`p` = with peak picking)

Proceed with additional experiments from step 3, or go to 'Finishing up Acquisition of Spectra'

Acquisition of ^{19}F and ^{31}P NMR Spectra (note: ^1H -NMR must be acquired first)

34. `jexp4` (go to experiment 4)

35. `f19cdcl` or `p31cdcl` (loads solvent-dependent parameters)

36. `nt=xx` (sets number of scans)

37. `ga` (starts acquisition) - wait for acquisition to finish (beep will sound)

Phasing and other processing procedures are exactly as for ^1H spectra; see steps 15 – 21, above.

Finishing up Acquisition of Spectra and Logging Out

38. `e`

39. reinsert cleaned (use Kimwipes) standard sample

40. `i`

41. `jexp1` (go back to experiment 1)

42. `standard` – wait for setup function to complete (beep will sound)

43. exit

44. In order to log out from the Sun computer click the **EXIT** button in the control panel on the bottom of the screen.

Supplementary Instructions

Miscellaneous Functions

- in order to change the chemical shift reference type `rl(x.xx*sfrq)` after placing the cursor on peak of interest; `x.xx` is the chemical shift value to be entered (e.g. 7.26)
- in order to observe a spectrum during acquisition type `wft` and process the spectrum as described above
- if the number of scans was not satisfactory repeat `wft` after additional scans
- if the spectrum is satisfactory type `aa` (abort acquisition) and process the spectrum
- `axis='h'` changes the axis unit to Hertz, `axis='p'` changes it to ppm
- if you would like to restart phasing with its initial values type `lp=0 rp=0 ds`, then repeat the phasing procedure

Storing and Retrieving NMR Data and Shim sets

- to save NMR data, use the following command: `svf('filename')`
- to retrieve previously stored NMR data use: `rt('filename')`
- for saving and retrieving shims files use: `svs('filename')` and `rts('filename')`
- after retrieving shims you must use the command `SU` to load the shims
- NOTE: filenames can include letters, numbers, dash and underscore characters **ONLY**

Adjustment of the integral trace

The **LVL/TLT** button activates interactive zero and first-order baseline correction mode. The zero order correction is represented by the **LVL** parameter; the first order correction is represented by the **TLT** parameter.

Position the cursor on an integral region of interest, about halfway vertically up the screen, and click the left mouse button. A horizontal line will intersect at the cursor and two vertical lines will be placed on either side of the cursor. Now moving the cursor above or below the horizontal line, but within the two vertical lines, and clicking the left or right mouse button will adjust the zero-order baseline correction parameter *LVL*. Placing the cursor right on the horizontal line and clicking the mouse button will restore the initial value of *LVL*.

Now move the cursor to another region of the spectrum, outside the vertical lines, and click the left mouse button again. A new horizontal line and two vertical lines will be displayed again and a single vertical line will be displayed in the middle of the region where *LVL* was being updated. The mouse will now control the first-order baseline correction parameter *TLT*. Clicking the left or right mouse button above or below the horizontal line will now increase or decrease *TLT*., and will also change *LVL* so that the total drift correction at the single vertical cursor in the middle of the previous region will be held constant. This process substantially reduces the necessity to iteratively adjust the two parameters *LVL* and *TLT*. As with the zero-order correction, clicking onto the horizontal baseline will restore the initial value of *TLT*.

Each time the cursor is moved outside the two vertical lines and the mouse button is clicked, a new vertical and horizontal line is displayed. The parameter adjustment alternates between adjusting *LVL* and adjusting *LVL* and *TLT*. The left and the right mouse button both adjust the baseline correction parameters and differ only in their sensitivity; changes with the left mouse button are eight times larger than changes caused with the right mouse button.

The middle mouse button adjusts the integral scale (height of integral trace). To exit the interactive baseline correction mode, type `ds` .

Using the sample temperature controller

Type `temp` to open the temperature control window. Using the left mouse button, adjust the slider to the desired sample temperature. Activate the temperature controller by clicking the “Turn temperature control on at...” button. Close the window using the pulldown menu in the upper left corner.

To turn off the temperature controller, simply open the window again (or leave it open during your experiment) and click on the “Turn temperature control off” button. Be sure to leave enough time for the probe to cool to room temperature prior to the next user starting his or her experiment.

This command will allow the sample temperature to be controlled at room temperature or above. The upper limit for sample temperature control is 100 degrees Celcius, or 10 degrees C below the boiling point of your solvent, whichever is lower. If you need to carry out low-temperature VT experiments, please consult with PINMRF staff for further training.

Varian 300 MHz NMR Spectrometers Parameter Files and Macros

h1acet	f19cdcl	p31cdcl	c13acet
h1cdcl			c13cdcl
h1d2o			c13d2o
h1dmso			c13dmso
h1meth			c13meth

h2cdcl (Inova-300-1 only – requires cables to be changed)

deptjsh (sets up DEPT-135 experiment from the current ¹³C experiment)

homodecjsh (sets up a homonuclear decoupling experiment from the current ¹H experiment)

noediffjsh (sets up a nOe difference experiment from the current ¹H experiment)

presatjsh (sets up a presaturation experiment from the current ¹H experiment)

cosyjsh (sets up a 2D-COSY experiment from the current ¹H experiment)

hetcorjsh(1) (sets up 2D-HETCOR experiment from the current ¹³C experiment and the ¹H experiment already run in experiment 1)

VNMR Commonly Used Keyboard Commands

Experiment Setup

jexpn - join (go to) experiment #n
 rt('abcd') - read NMR data file "abcd"
 rtp('abcd') - read parameter set "abcd"
 rts('abcd') - read shim set "abcd"
 svf('abcd') - save NMR data file "abcd"
 svp('abcd') - save parameter set "abcd"
 svs('abcd') - save shim set "abcd"
 mf(x,y) - move NMR data (FID) from experiment "x" to experiment "y"

Data Acquisition

h1xxxx - load acquisition and lock parameters for ^1H experiment in solvent xxxx
 (xxxx = acet, cdcl, d2o, dmsd, meth)
 acqi - open lock / shimming window
 su - set up interfaces
 gain=xy - set receiver gain to value "xy" ("xy" = 1 – 60)
 nt=xy - set number of scans to value "xy"
 bs=xy - set block size to "xy" scans
 ga - zero current data and start acquisition, Fourier transform and display result
 go - zero current data and start acquisition
 aa - abort data acquisition after next scan
 standard - set up spectrometer for CDCl_3 standard sample
 f19xxxx - load acquisition parameters for ^{19}F experiment in solvent xxxx
 p31xxxx - load acquisition parameters for ^{31}P experiment in solvent xxxx
 c13xxxx - load acquisition parameters for ^{13}C experiment in solvent xxxx
 deptjsh - set up DEPT-135 experiment from current ^{13}C experiment
 homodecjs - sets up a homonuclear decoupling experiment from the current ^1H experiment
 noediffjs - sets up a nOe difference experiment from the current ^1H experiment
 presatjs - sets up a presaturation experiment from the current ^1H experiment
 cosyjs - set up 2D-COSY experiment from current ^1H experiment
 hetcorjs - set up 2D-HETCOR experiment from current ^{13}C experiment

Data Processing and Plotting

text('abcd') - set title for plot to be the text string "abcd"
 ft - Fourier transformation
 wft - exponential multiplication and Fourier transformation (can use after bs scans completed)
 lb=xy - set amount of exponential multiplication equal to "xy"
 aph - automatic phase correction
 rl(x.yz*sfrq) - set chemical shift at location of cursor to x.yz ppm
 th - set threshold for peak picking using mouse
 dpf - show peak labels
 ds - re-display spectrum with cursor active
 dir - show a listing of the current directory
 print, printp, printi, printpi - print spectrum with/without peak labels (p) and/or integration (i)
 print135, print135p - print DEPT-135 spectrum with/without peak labels (p)

**Checkout Practical Exam Requirements -
Varian 300 MHz NMR Spectrometers
PINMRF**

Prior to performing this practical exam you must first pass a written exam. The written exam will test you on material in this document and the Facility Overview document, as well as spectrometer-related matters.

This is an outline of the tasks you will be required to perform correctly in order to pass the checkout practical exam. You will be allowed up to 30 minutes to complete the following tasks. You may use any training handouts and notes you have. Standard samples will be used for the checkout and will be provided by the NMR lab. PINMRF staff may, at their discretion, observe you performing all or part of the checkout exam.

Test Sample - 10% Ethylbenzene in CDCl₃ (or other sample as required)

- login to computer and start VNMR
- remove CDCl₃ standard, insert new sample, lock and shim
- run ¹H spectrum, process, integrate and plot
- set up new experiment for ¹³C spectrum
- run ¹³C spectrum, process and plot

Standard Sample - 100% CDCl₃

- remove test sample and replace with CDCl₃ standard sample, lock and shim
- initialize spectrometer to ¹H
- shutdown VNMR and logout from UNIX