Varian Solution NMR Procedure

(VNMRS Machines running VNMRJ 3.2 under Red Hat Enterprise 5.1)

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**Safety Issues**

⚠️ If you, or people working with you, have magnetic metal implants, please consult your doctor for possible effect of magnetic field;

⚠️ For those who have pacemakers, please stay away from NMR magnets;

⚠️ Remove from your pocket anything ferromagnetic or vulnerable to magnetic field:
  - Your wallet, bank cards, credit cards, and any cards with magnetic stripes;
  - Electronics: cell phone, mp3, ipod, etc.;
  - Mechanic watches;
  - Keys and other magnetic items.

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I. Sample preparation

1. NMR tubes of **5mm** in OD and **8”** long are used and available from:
   i. Chemistry Department Stockroom, UCSB (Phone: x2107)
   ii. Aldrich (Phone: 800-558-9160)
   iii. Wilmad (Phone: 800-220-5171)
   iv. Newera (Phone: 800-821-4667)

   Please get tubes for 600MHz or higher.

2. Samples are dissolved in **deuterated** solvents (~0.1 mM and 50 mM in concentration for $^1$H and $^{13}$C, respectively, and at least 0.7 ml in volume or 56 mm in height) for three purposes.
   i. Deuteration removes solvent $^1$H signals which would otherwise dominate the $^1$H spectrum.
   ii. Deuterons provides a lock signal.
   iii. Deuterons provide chemical shift reference for NMR spectra, rendering addition of reference standards such as TMS unnecessary.

   ![Tip] **Label your samples with your name and your advisor’s name. This helps us take care of unknown samples.**

II. Sign onto Logsheet

Enter
1. your name
2. your advisor’s name and department
3. your recharge account number (in the format: 8-4xxxxx-xxxxx-3)
4. your start time
5. (Do this at the end of experiment: your stop time and duration of experiment)
6. (Do this at the end of experiment: Status of instrument and report problems if any)

III. Start VNMRJ Software

1. Make sure that the spectrometer is idle by looking at the computer. If yes, proceed to Step 2 below (if no, either wait, talk to the user, or do something else)
2. Login into your NMR account:
   Type your username, hit the Enter key
   Type your password, hit the Enter key again
3. Double click on the VNMRJ icon on the desktop, the last VNMRJ layout from your previous login session will appear.

IV. Preparation: Load Samples onto Auto-Sampler

1. Put your sample in a green spinner, measure depth with the golden depth gauge or the rack for spinners, and clean the bottom half of the sample tube with a napkin while holding the top half of the tube;

2. (Important information) Slot 12, aligned with the yellow triangle label at the bottom front of the sampler, is reserved for the standard idle sample (pure CDCl₃). Don’t load any sample into this slot.

   The standard idle sample will be loaded into the magnet automatically at the end of an automation run.
3. Go to the magnet and load your samples onto the sampler in the clockwise order: the 1\textsuperscript{st} sample goes to slot 1, the 2\textsuperscript{nd} to 2, the 3\textsuperscript{rd} to 3, and so on.

Be very careful to align the sample tube with the slot holes when removing or loading samples.
V. 1D $^1$H and $^{13}$C NMR Setup and Data Acquisition

1. In the Review mode (where you don’t see the button. If you do, click on it to switch to Review mode), give a name (Letters and Numbers ONLY plus underscore _). No spaces and special characters) to the new automation run in the Sample Name box on the Start/Standard page:

   ![Sample Name: metabolism]

2. Choose New Automation Run from the Automation menu to create a new automation session: (this resets the StudyQ panel and the tray display)

3. Click on in the StudyQ panel to go to the Submit mode.

4. In the Horizontal Panel, click tab to show the Start/standard page below.

![Start/standard page]
• Fill out the **Sample Name** box with a meaningful name for the sample (Letters and Numbers ONLY plus underscore _. No spaces and special characters).

• Choose the **Solvent** your sample is using.

• Record a meaningful title in the **Comment** box for the sample for future reference.

• Check or uncheck the boxes for autolock, autotune, and autoshim as necessary:

  - When aqueous solvents are used, or
  - When run any other nuclei than $^1\text{H}$, or
  - When salt concentration is high, or
  - When running variable temperature (VT) experiments.

If you want to change the information on the **Start/standard** page later on, click on **SampleInfo** in the StudyQ panel:

5. Select experiments with left-clicks from the lists under **Experiment Selector** in the vertical panel of VNMRJ window. For example **Std 1D** for 1D NMR.
to populate the Study Queue, which looks like the figure below after "Proton" and "Carbon" are selected.

<table>
<thead>
<tr>
<th>Study Queue</th>
<th>Study Queue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submit Queue</td>
<td>Done</td>
</tr>
<tr>
<td>5p_Ethylbenzene_T (to be submitted)</td>
<td></td>
</tr>
<tr>
<td>SampleInfo [Day:11:04]</td>
<td></td>
</tr>
<tr>
<td>PROTON_001_day [0:24]</td>
<td></td>
</tr>
<tr>
<td>CARBON_001_day [8:40]</td>
<td></td>
</tr>
</tbody>
</table>

Drag an item to delete.

If other 1D NMR’s and 2D NMR’s are to be run, do basically the same thing.

6. Double click on node "PROTON_001_day [0:24]" in the Study Queue to load parameters for \(^1\)H. If successful, you will see a new graph in the NMR Data Display area and the pages associated with the "Acquire" tab in the Horizontal Panel, as shown below.

Change "Number of Scans" and "Relaxation Delay" as necessary by clicking on the "Acquire" tab and then on "Default H1".

**Acquire/Default H1 page**
7. Do the same for $^{13}$C: double click on node **CARBON_001_day[8:40]** in Study Queue to load parameters for $^{13}$C.

8. In the Horizontal Panel, click on the Acquire tab and then on Default $^{13}$C to show the default $^{13}$C parameters. Change **Number of Scans** and **Relaxation Delay** as necessary.

9. If **Number of Scans** has to be set to a large number, e.g. 128 or higher, make sure **Save FID at each block** is checked and set on the Acquire/Flags page. Typical Block size is 32 or 64.

10. Click on **1** at the top left corner of the Graphics Display area and then on the numbered circle where the 1st sample is located (slot 1) in the tray display to have it selected: (**1**: and **and** toggles the tray display and NMR data display)
11. Click on the Submit button to start data acquisition. The auto-sampler will start changing samples by lifting up the CDCl3 standard from the magnet, rotating counter-clockwise, and loading the sample in the 1st slot to the magnet. Followed by auto-tune, auto-lock, gradient-shim, and data acquisition.

Color codes in the tray panel:

- **Sample Active**
- **Sample in Queue**
- **Sample Finished**
- **Sample with Error**

- To stop a running experiment and move on to the next one in the queue, use Stop-Save-Resume or Stop-Discard-Resume under the Automation menu (click Done if the menu is not active).
- To abort a whole automation run, use Abort Automation and then Resume Automation (or the machine will hang in the automation and nobody can use it) under the Automation menu (click Done if the menu is not active).

12. While waiting for all experiments for Sample 1 to finish, you may setup the experiments for Samples 2, 3, etc. by clicking on Done as necessary and then repeating steps 3 to 11 above to create a new StudyQ for a new sample.

13. To view progress of experiments or process the finished experiments, click on Done as necessary and choose View: Spectrometer or View: Active Sample.
Same color codes as in the tray display: finished, running, and waiting.

You don’t have to wait until the whole StudyQ (sample) finishes in order to process data. You can process the Proton data while waiting for Carbon to end, for example.

To toggle between the normal graphics display and the tray display, click on in or near the top-left corner of the graphics display area.

14. At the end of the Automation Run, the sample tray will rotate multiple times to put slot 12 in the middle and the standard idle sample will be placed in the magnet automatically.
VI. Finishing up

⚠️ YOU ARE NOT FINISHED WITH THE SPECTROMETER UNTIL YOU DO THE FOLLOWING.

a. Set solvent to CDCl$_3$ on the **Study/Standard** page as shown:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Chloroform</th>
<th>DMSO</th>
<th>D$_2$O</th>
<th>other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>CDCl$_3$</strong></td>
</tr>
</tbody>
</table>

b. Click on **Find z0** on the **Start/Standard** page and wait for finish:

```
Lock & Shim
Tune
Find z0
Gradient Autoshim
```

Start/Standard page

```
4W008_5mm_DB_Lk_2011-04-19
```


c. Exit VNMRJ by going to **File** menu and choosing **Exit VNMRJ**.

d. Log off your account: in the bottom left corner of screen click on the logout button.

```
System
```

and hit **Log Out** in the popped up window.

e. **Important:** On the logsheet, record your stop and duration times, and the spectrometer status. Report problems if any.

f. Remove your samples from the lab and clean the space you have used.
VII. Data Processing Workstation

- Please go to the three data processing computers and use the computer in the MIDDLE.

1. Login
   a. Type “vnmrp” as a username and input password “4epp967”

2. Click “Home_600MHz” icon on the desktop to access files

3. Open “VNMRJ 2.3” software on desktop

VIII. NMR Data Processing

1) Load your NMR file:

   Choose “Open” → Your username → Your experiment → Open the .FID file (Not the folder!)

2) Click on near the upper right corner of VNMRJ to show the full graphics toolbar, if not shown, for display manipulation
3) Phase the spectrum:

   a) To better see the phase, scale up the spectrum vertically using `+` on the Process/Display page. ` Autoscale` and ` -` are for normalization and scaling down.
For continuous vertical scaling, click-and-hold the middle-mouse-button on the top of a peak and move the mouse up and down.

For horizontal scaling, use buttons in Graphics Tool Bar.

For horizontal shift, use in Graphics Tool Bar.

b) Click on in Graphics Tool Bar and then on the rightmost region of peaks on the spectrum. The region (~2 ppm wide) will be marked with two vertical red lines and one horizontal red line as below:

c) Place the mouse cursor on the horizontal red line between the two vertical lines, click-and-hold the right or left mouse button and move the mouse back and forth for fine or coarse phase adjustment, respectively, of the phase for the rightmost peak(s).

d) Once the peak(s) are phased, click on the leftmost region of peaks (~2 ppm wide) and use the right or the left mouse button for phase adjustment of the leftmost peak(s).
e) Repeat steps b) to d) if necessary.

f) After phasing, click on in the Graphics Tool Bar to refresh spectrum display and on on the Process/Display page to normalize the spectrum if necessary.

4) Baseline correction for accurate integration: on the Process/Display page (see layout above), click on to correct the baseline DC offset and then on to get rid of the curvature or wobbling of the baseline.

5) Integration: click on to go to the Process/Integration page:

For horizontal scaling, use buttons in the Graphics Tool Bar.
For horizontal shift, use in Graphics Tool Bar.

- Make sure is properly checked.

- Click on to clear automatic integrals and all peaks are integrated as a whole:

- Click on and left-click on both sides of a region to be integrated. Repeat left-clicks on other regions of interest.

- To show integrals on spectrum, click on .

  To show the list of integrals in the Horizontal Panel, click on .

  To calibrate the integrals to a single peak:
- Go to activate the single-cursor mode by clicking on \[\text{cursor} \] in the Graphics Tool Bar so that you see a vertical red line on the spectrum.
- Click on the integral you want to calibrate.
- Check radio button \[\text{Single Peak} \] on the \textbf{Process/Integration} page.
- Type a value in the box next to \textit{Integral Area}.
- Click on \textit{Set Integral Value}.
- Click on \textit{Show Integral Values} to show integrals on the spectrum.
- To plot the calibrated values, select \textit{Scaled, Vert.} on the \textbf{Process/Plot} page.

6) Peak Picking:

i. Click on \textit{Peak Threshold} on the \textbf{Process/Display} page to show the threshold (a horizontal yellow line) on the spectrum. Only those peaks taller than the threshold will be picked up.

![Peak Threshold](image)

ii. Place the mouse cursor on the yellow line and use the left-mouse-button to drag the line up or down.

iii. Click on \textit{Find Peaks} on the \textbf{Process/Display} page to label peaks with their chemical shifts on the spectrum.
7) Print spectrum:

   a) Select a region to print: click on in the Graphics Tool Bar, go to spectrum, click-and-hold left mouse button and drag to zoom-in the region to be plotted. Use on the Process/Display page to normalize the spectrum.

      For more horizontal scaling, use buttons in Graphics Tool Bar. Or go to the two-cursor display using , put the cursors where you want, and then click on .

   b) Click on to show the Process/Plot page:
Process/Plot page

Step c

c) Click on **Automatic Plot Page** to print out the spectrum with integrals and chemical shift labeled.
IX. Appendices

1. Requirements for Access to the MRL NMR at CNSI

You have to pass the mini quiz within one month after training in order to be qualified for access to the NMR facility of MRL, which includes:

- Key Card for Lab & Building:
  1. Pass the MRL safety training;
  2. Fill out the CNSI access form: [http://www.cnsi.ucsb.edu/facilities/building_services/access/access_application.pdf](http://www.cnsi.ucsb.edu/facilities/building_services/access/access_application.pdf)
  3. Take the form to Sylvia in 2066G, MRL
- Web Scheduling Account
- NMR Account

These requirements apply to both on- and off-campus users.
X. NMR Basic Principles

1. Spin

*Spin is a quantum mechanical phenomena that has no physical analog in classical physics. However, it will be helpful to visualize it as a small bar magnet that precesses about an axis.

*The existence of spin angular momentum is inferred by experiments, such as the Stern-Gerlach experiment, in which particles are observed to have angular momentum that cannot be solely accounted for by orbital angular momentum alone.

*Electrons, protons, and neutrons all have a value of spin +/- \( \frac{1}{2} \).
2. Common NMR Nuclei

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Unpaired Protons</th>
<th>Unpaired Neutrons</th>
<th>Net Spin</th>
<th>$\gamma$ (MHz/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1\text{H}$</td>
<td>1</td>
<td>0</td>
<td>1/2</td>
<td>42.58</td>
</tr>
<tr>
<td>$^2\text{H}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6.54</td>
</tr>
<tr>
<td>$^{31}\text{P}$</td>
<td>1</td>
<td>0</td>
<td>1/2</td>
<td>17.25</td>
</tr>
<tr>
<td>$^{23}\text{Na}$</td>
<td>1</td>
<td>2</td>
<td>3/2</td>
<td>11.27</td>
</tr>
<tr>
<td>$^{14}\text{N}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3.08</td>
</tr>
<tr>
<td>$^{13}\text{C}$</td>
<td>0</td>
<td>1</td>
<td>1/2</td>
<td>10.71</td>
</tr>
<tr>
<td>$^{19}\text{F}$</td>
<td>1</td>
<td>0</td>
<td>1/2</td>
<td>40.08</td>
</tr>
</tbody>
</table>

Larmor Frequency Equation:

$$\nu = \gamma B_0$$

where $\gamma$ is the gyromagnetic ratio (specific to each nuclei) and $B_0$ is the magnetic field strength

3. Energy Level Diagram
4. cw NMR

Alignment of nuclei in a magnetic field

5. Magnetization
6. Pulsed NMR, Relaxation, and Detection