

**Throughout**: when you have to click on a 'button' the button's name is outlined, when you have to type a command the command is in **bold** and when I refer to a parameter it will be underlined.

**Practicum 1, Spring 2005**

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### **Collecting a basic 1d spectrum.**

We will go through basic steps in maintaining your account, loading a sample, locking, shimming, collecting a simple 1d spectrum, processing it, saving it ejecting the sample and leaving the machine for the next user.

#### **Log On.**

Log on to the Computer. Our course account name is che555 and the password is nmr4you. Don't change the password please.

Open a terminal shell.

Type **ls** to see the contents of your 'home' directory. (bold face text is a command to be typed in, followed by the return key).

**cd data** to enter the data subdirectory where you should store all the spectra you wish to save.

**mkdir <yourname>** to create a subdirectory of data for your saved spectra (the <> brackets indicate that you should replace the brackets with your name or initials, i.e. actually type 'mkdir afm'. We ask each of you to store your spectra in your own data subdirectory, in order to reduce the confusion that arises from many saved spectra with different naming systems. Establish a naming system for yourself that facilitates identification of stored spectra with notes and sample identities. For example include a reference to the sample and the date in the file name.

**cd ..** takes you back up one level in the directory tree to the common data directory.

Put away the terminal window.

Launch vnmr by clicking on the icon. It will open in the last experiment that was active, which may not be the same as the last experiment YOU ran.

#### **Get Oriented**

**explib** to list the contents of all the experiments available to you.

**jexp4** to join experiment number 4 if that one is set up to do approximately what you want to do, or contains a no-longer-necessary experiment which you can over-write with what you want to do. (4 is just given here as an example.) Since we will all share the same class account save anything important to you. experiments will be overwritten regularly.

**dps** (display pulse sequence) to display the pulse sequence currently set up and

**dg** to 'display group' display the group of parameters to be used.

#### **Load a Sample**

**acqi** (click on the acqi button) to activate the interactive acquisition window.

Check the condition of your sample, tube etc. Only put intact, clean tubes in the spectrometer. Check that your solvent is compatible with the temperature of the probe. Please avoid touching the black belt or white dots on the spinners, there are used by the tachometer and cannot be read if they get too dirty or worn.

**eject** to eject the current sample, go and get that sample.

Dry the outside of your tube. Mount your sample in the spinner, ensuring that it is securely held, and positioned where the probe is most sensitive (using the depth gauge). You are encouraged to always try to have

the same volume of sample eg. 600  $\mu\text{l}$  or 750  $\mu\text{l}$  (5 cm or more is ideal), so that your sample height and thus shimming will be consistent. Remember that at least 10% of your solvent must be deuterated at a single position.

Place your sample at the top of the magnet so that it floats in the lift air stream.

insert to lower your sample into the magnet.

### **Lock and Shim Crudely on your Sample**

lock Adjust the zo to minimize the frequency of the beats between the actual  $^2\text{H}$  resonance frequency and the  $^2\text{H}$  lock transmitter.

Adjust lock power to approach but not reach saturation, for maximum shimming responsiveness. A decrease of the power by 4 should be compensated well by an increase in the gain by 4.

shim adjust the z1 correction to the field such as to maximize the 'lock level'. Then proceed to z2, return to z1 and alternate until the optimum combination is obtained. In the bad old days you would then proceed to include z3 and z4 in the optimization cycle, then z5 and z6 as well (see Claridge's book).

close out of the acqi window.

Check alock and wshim. these should be set to 'n' unless you wish the instrument to automatically override your settings and find new ones.

### **Begin Setting Up**

In your chosen experiment, set up an NMR experiment the simple way by loading Varian's default parameters (a good place to start, not perfect but 'good enough for government work'). Click on main menu, set up, nucleus, solvent, H1, CDCl<sub>3</sub>. The different spectrometers have capabilities of observing different nuclei, but all can collect  $^1\text{H}$  spectra. One of the Gemini also collects  $^{13}\text{C}$  and thing 1 is set up for  $^{19}\text{F}$  and  $^{31}\text{P}$  as well. For more options you will have to use Thing 2 with John Layton's help.

Check that the temp parameter is turned off by typing **temp ?** to 'ask' VNMR what temp's current setting is. The response should be 'n'. If it is not set temp to no (temperature regulation off): **temp = 'n'**.

Type **su** to 'set up' i.e. send the parameters on your computer screen to the NMR console.

type **gshmsr** (gradient shimming start) to initiate the gradient shimming process. This launches a small program of automated events (a macro) that will optimize the z shims for most samples and most conditions. Special cases may require more user intervention, and will be covered later. (\*\*This macro is specific to the UK NMR centre, and not generally available).

Click on the button autoshim on z

When the computer obtains shims it finds acceptable, it will tell you so and stop making further adjustments.

Type **gshmend** (gradient shimming end) to quit the gradient shimming routine and return to the experiment you are setting up.

Having done this, make sure that alock = 'n' and wshim='n', so the machine will not attempt to redo your shimming etc.

### **Check Experimental Parameters**

**dps** to check that the pulse sequence to be executed is indeed what you want. ALWAYS do this before starting an experiment. It takes 2 seconds and can save a \$30,000 probe. We intend to apply a single short 'observe' or 'probe' pulse that should not exceed pw = 30  $\mu\text{s}$ . This should be followed by an acquisition period (acquisition time, at) during which the detection coils will measure and the computer will digitize the magnitude

of signal, as a function of time. For 'small' molecules of molecular weights less than  $\approx 1000$  Da at should be more than 1 s long. All of this is preceded by a relaxation delay 'd1'. We most often want to signal-average over several scans to improve signal-to-noise and cancel spectral artifacts (a later lecture). The d1 interval serves to allow magnetization excited by one pulse to recover before the next is collected. Note that because we are performing Fourier transform NMR we are not in fact scanning at all, but the term persists from the days of 'cw' field-swept NMR. For at = 1 set lb = 1, for at = 2, set lb = 0.5 etc.

**dg** to display the other parameters that will control your experiment.

nt indicates the number of transients that will be averaged together. We have the luxury of a nice pure concentrated sample, so we only need nt = 1.

ss is the number of times the pulse sequence will be executed beforehand, without any data being collected. Such 'warm-up' runs establish an equilibrium for the magnetization in the context of your pulse sequence. This will become more important later. For simple observation of a single  $^1\text{H}$  spectrum ss can be 0.

It is worth taking a minute to check that the transmit nucleus 'tn' is in fact the nucleus you requested, i.e. H1. 'tof', the transmitter offset, determines the frequency at which your spectrum will be centered, like the setting on your radio dial. 'sw', the sweep width, indicates how wide a frequency window will be observed. For a first look at a new sample, sw = 6000 is a good choice on a 400 MHz spectrometer, sw = 3000 is a good starting point on a 200 MHz spectrometer. These are chosen to be much wider than the spectrum is likely to be, so that no signal will be missed. The sw can be optimized later, once you have a spectrum to look at.

Check the gain being used to amplify the signal prior to digitization by typing **gain** ? If you have proteated solvent you must set gain = 0 until you have solvent suppression working (later lecture). With a deuterated solvent and a strong (concentrated) sample, gain = 20 is a good starting point.

Finally, for the good of the nation (the spectrometer probes) check that the decoupler is off. This was also evident in the dps window, but the parameter responsible, 'dm' or decoupler mode, should be set to dm = 'n'.

### **Collect a trial Spectrum and adjust its Phase**

Type **ga** to execute the experiment. You should see a green light flash indicating that a pulse was successfully executed. You should see the yellow 'acquire' light come on for the 2 second acquisition period. You should NOT see the red overload light come on (ever). If it does come on, decrease the gain (**gain** = 10) before trying again.

Use of the **ga** command automatically results in weighted transformation of your data into a spectrum (Fourier transformation will be discussed next week and weighting will be discussed thereafter).

The functions of the three mouse buttons depend on what menu you are working under, and are shown below the spectral window. When a spectrum is being displayed in interactive mode, depressing the middle mouse button allows you to adjust the vertical scale of the spectrum. A short click will adjust the scale to bring the spectrum's height to the position of the mouse at the frequency of the cursor. Depressing and dragging the mouse up and down expands and shrinks the spectrum vertically. clicking at the left-hand edge repositions the spectrum vertically. Practice makes close-to-perfect. The left-most mouse button places and moves a cursor, allowing you to read spectral amplitude. The right mouse button allows you to define the width of a 'box' or spectral region. The screen can be toggled between box and cursor mode (two cursors and one) using a menu button.

Your spectrum may be dispersive. You can have the computer correct this by issuing the **aph** command (automatic phase adjustment), which works pretty well. You should however also be comfortable with adjusting the phase manually. Click on the phase button in the menu, this changes the functions of the left and right mouse buttons, but not the middle one. Move the cursor to the right-most resonance in your spectrum. Depress the left button and drag up or down to untwist the signal and produce an absorptive line with a symmetric 'balanced' baseline on either side. Release the mouse button and move the cursor to a signal near the left end of the spectrum. Depress the left mouse button again and adjust the phase of this signal. When you are

done click on the **phase** button again or on the **box** button. The right mouse button acts similarly to the left mouse button but applies finer control to the phase.

To zoom in on the spectral region containing your spectrum place cursors on either side of it and click on **expand**.

Many commands can be used to modify the display and annotate it (below). A few particularly useful ones are **f** (show the full spectrum), **full** (use the full screen width), **s1** (s2, s3, . . . , save the current expansion), **r1** (return to the expansion saved with s1).

The vertical expansion of your spectrum is achieved via the middle button of the mouse. Either click one at the height you would like a particular position of the spectrum to have, or 'grab' a position on the spectrum and drag it up (with the mouse button depressed). Alternatively, you can specify the value of the vertical scale (**vs**) parameter, eg. **vs** = 100, and use mathematical commands to increase or decrease the amplitude of the spectrum (eg. **vs** = 4 \* **vs**). **vp** controls the vertical position (offset). You can enter a numerical value (**vp** = 30) or modify it interactively by dragging the mouse with the middle button depressed and the cursor at the left edge of the screen. Before doing this though, type **dc**, to introduce a drift correction to the data and in essence place it at a **vp** of 0.

**dscale** displays the scale, **dscale(0)** displays it at a vertical height of 0, or whatever you entered in the parentheses. The scale will be in ppm if **axis** = 'p' or in Hz if **axis** = 'h'. To calibrate the scale place the cursor on the top of a line of known chemical shift (eg. TMS at 0 ppm) and type **rl**. If the reference line chosen is not at 0 ppm, for example it is at 10, you have to specify that using **rl( 10p )** instead of just **rl**. Now **dscale** again to see the correct chemical shifts of all your lines.

To collect a spectrum containing only the spectral regions containing your spectrum expand around your spectrum so that it fills the display with  $\approx 10\%$  of the spectral width allowed for baseline on either side. Then type **movesw** (move sweep width). The computer will calculate new values for **tof** and **sw**, while retaining spectral calibration information.

### Collect a 'Good' Spectrum and Obtain Peak Positions and Integrals.

Set **nt** = 8 **ss** = 2 and **ga** to collect a new spectrum of the correct width. You will have to adjust the phase again, as above. Even if you don't need 8 scans for sensitivity reasons, averaging 8 scans will cause a couple of artifacts to cancel out of the spectrum (a later lecture). This doesn't take much time but is good practice.

Correct the baseline: display the spectrum in interactive mode (**ds**) and click the **full/partial/no integrals** button until it you are displaying partial integrals. **cz** to clear any pre-existing selection points. Choose the menu option **resets**. Working from left to right, place the cursor on the baseline immediately before the first signal and left-click once, then move the cursor just past the signal and click again. You have now delimited that signal. Repeat for each of the signals in the spectrum. You may choose to group some clustered signals together, especially if they represent a single split signal. You can remove a misplaced selection point by clicking with the right mouse button.

You should now have dotted green integral line between signals and a solid green integral line arching up at each signal. Typing **bc** (baseline correct) will cause the computer to use regions between signals as the basis for calculating a baseline, which it then subtracts from the spectrum to yield a spectrum vertically positioned with the baseline at 0. (Alternately you can **dc** (drift correct) to position the spectrum at 0 without manipulating the baseline.)

The heights of the individual integral lines now represent the areas under the peaks to which they correspond. These areas can be displayed several ways. **dli** lists them **dpir** presents them in the display **dpirn** does so and normalizes to the largest signal in the spectrum. To use the latter two options the spectrum must be positioned vertically with **vp** = 12 or more, so that the program has space to print the integral values beneath the axis. Very small integrals will simply appear as 0.00. set **ins** to a large value, use normalized integrals, and

*list* (**dll** or **pll**) instead of *displaying* integrals to get values for small ones. If you want to use a particular integral as the reference, and have its value be a particular value, then do **dpirn**, note the actual value of the reference integral and set **ins** to **ins = ins\*<the desired value of the integral>/<the current value of the integral>**. (If the integral currently has a value of .06 but you want it to have a value of 1, type **ins = ins\*1/0.06**). **ds** and **dpirn** again and the reference integral should have the value 1 (or the desired value). Recall that the resonance of one proton may be split into several lines by J coupling !! Integral display can be turned off again using the **full/partial/no** integral button.

Other useful commands: **dprf**

Peak positions can also be listed or displayed, either before or after correcting the baseline and integrating. In interactive display mode, click on the **threshold** button. A horizontal line will appear. Position this using the mouse so that it is below the tops of signals you wish to list but above the tops of baseline teeth and rolls (and signals of impurities you do not want to draw to your advisor's attention). Typing **dprf** (display peak frequencies) will now result in all the taller-than-threshold signals being identified with the chemical shift (when axis = 'p') or frequency (when axis = 'h'). **dll** lists peak frequencies. As for the integral display commands, there are lots of variants of these, and you will have to experiment to find the one you prefer. John Layton has written macros that provide custom options that are convenient for both purposes. Line lists have many uses, to be discussed later.

### Annotate and Print Your Spectrum

Annotate your spectrum using the parameter/command text and the command **atext**. Type **text('my very first spectrum at UK')** to give the string parameter **text** the value my very first spectrum at UK. You can now record things like the identity of the sample, or interesting observations in the text line. More text can be entered using **atext('baseline wavy because I collected these data while PPD was using a jack-hammer 2 feet away')**. (Please call John Layton, Dr. Meier or Dr. Miller immediately if anyone ever suggests they will try this or other similar lunacy.)

To print your spectrum type **pl**. Most commonly you will type a string of printer-related commands to plot the spectrum (**pl**), also plot its ppm axis (**pscale**), include the integrals (**pir** or **pirn**), include line positions (**ppf**) and parameter information (**pap** or **ppa** provide two different formats). All of this goes to a virtual page called a print buffer but is not actually set to paper until you issue the command **page**. Thus, a typical print order might be **pl pscale(0) pap(200,160) page**. John Layton has written macros to simplify your life in this area too, **plt** plots the spectrum and the axis to a page. We advise against using **pll** on the same page as **pl** etc, as this can result in congestion.

### Save, Save, Save

If you ever want to see your spectrum again, for example to include it in a document, save it as a file in your data directory. Click on the **main menu** button (whenever you need to get back to the top of the command tree). Then click on **file** to display the contents of the current directory. The **data** button will take you straight to the data directory and display all the subdirectories. Select yours and click on **change directory** to enter it. Now save your file: **svf('a\_useful\_filename')**. Personally, I like to include a date and information about the sample and pulse sequence if it is variable. To get out of a directory, click **main menu**, **file**, **change directory**, **parent**.

### Leave the Spectrometer Locked for the Next User

Replace your sample with the lock sample, and leave the machine locked and at least roughly shimmed (z1, z2) as above.

Exit VNMR using `main menu`, `more`, `exit`, or by typing **exit**. Give the computer several seconds to shut down VNMR, then log out by clicking `exit` in the SUN tool bar.

Before leaving either of the 400 MHz spectrometers, BE SURE to fill out the log book with your name, the solvent you used and any observations you had related to spectrometer performance. These are very valuable to John Layton when he is trying to diagnose weird, and especially sporadic, behaviour.

Remember when handling spinners to not touch the black belt around the top. Getting gunk on this reduces the contrast between the black background and the white dots, and makes it more difficult for the spinner's tachometer to read.

### **IMPORTANT**

Everyone breaks a sample in the machine at some time. IT IS VERY IMPORTANT THAT YOU REPORT THIS to John Layton (preferred) or Dr. Miller RIGHT AWAY. Breakage of a tube is regarded as human error, unless it happens regularly, but failure to report a problem or worse yet attempts to disguise one, are regarded as unprofessional abuse of our very fine and expensive shared research resource. Abusive users will not be allowed to use the spectrometers. Please report problems right away so they can be fixed while they are small, and before they result in more, avoidable damage.