NUCLEAR MAGNETIC RESONANCE RESEARCH RESOURCE

Low Temperature NMR on the AV-300

Dr. Mike Lumsden
Coordinator, NMR-3
October, 2014: version 1.7

SECTION A: LOW TEMPERATURE NMR RULES & RECOMMENDATIONS

Facility Rules Concerning Low Temperature Experiments

- Consider the freezing point of your solvent relative to the temperatures you wish to probe. As a general rule, stay at least 10° above the freezing point.
- Low temperature NMR experiments are by default only permitted during evenings and week-ends. Since this is typically walk-up (automation) time, you are required to notify Mike a day in advance of when you wish to perform your low temperature experiments.
- You are required to book an extra ½ hour of "dead time" at the end to allow thermal equilibration of the probe with normal operating temperature (300 K).
- Low temperature NMR experiments must be performed with the autosampler carousel removed and with the black, plastic cap in place over the bore of the magnet (where the sample drops into the magnet).
- Use must use the white ceramic spinner whenever you intend to go below 223 K.
- If you are studying two different samples at low temperatures, you will need to eject a cold sample and spinner assembly at some point in order to exchange samples. This creates the possibility of moisture condensing on the cold spinner, potentially problematic if inserted back into the magnet (i.e. ice formation). Since the facility owns two low temperature ceramic spinners, use them both to avoid this potential problem; the second spinner will be at room temperature and dry.
- If you are studying three or more samples, you must always ensure the spinner being used is warm and dry before inserting into the magnet. You can warm and dry a cold spinner by using a hair dryer available within the facility (remember the hair dryer should never be brought into the vicinity of the magnet).
- Spinning is not permitted with low temperature experiments.

Facility Hints & Recommendations Concerning Low Temperature Experiments

- Whenever possible, it is highly recommended that you collect room temperature data before beginning your low temperature experiments (to ensure your sample is what you assume it is).
- After obtaining an initial room temperature spectrum, you should lower the temperature to the lowest temperature you think you'll need. If you want to run spectra at several different temperatures, always start low and work your way back up towards room temperature.
- Due to reduced solubility at lower temperatures, the possibility of precipitation is an important concern. Thus, do not saturate your solution when preparing it at room temperature! Precipitation will cause significant line shape distortions which cannot be eliminated by shimming. A sudden drop in the lock level can indicate precipitation has occurred.
- Although high gas flow rates are required to achieve the lowest temperatures (and generally desired for tight temperature regulation), be aware that there exists the possibility of supplying too much gas flow such that the sample can be partially lifted upwards out of the probe. If you observe a sudden drop in the lock level (or lose lock), your sample may have partially lifted out of the probe. Under such circumstances, you will have to reduce the cooling power.

- If your sample must be kept cold while the probe is being cooled for low temperature NMR, the probe can be pre-cooled to the desired temperature without a sample in the magnet. However, moisture must not be condensed on your cold sample tube!
- A refill port is available for replenishing the liquid N_2 supply during lengthy low temperature experiments such that the heater does not need to be removed. Before replenishing the liquid N_2, first turn off the nitrogen heater for a short period before refilling to reduce the pressure in the dewar.
- The o-rings in the white ceramic spinners tend to be tight so exercise extreme caution when inserting your NMR sample tube into it.
- When cold, ceramic spinners are brittle and can be easily broken if dropped or handled roughly. Replacement cost is now on the order of $700!

Temperature Limitations

- The AV-300 temperature controller and associated hardware are capable of producing temperatures from 200°C down to -150°C with a stability of +/- 0.1°C. However, the NMR probe limits the operational temperature range from 150°C to -150°C.
- Spinners: the plastic blue spinners that are used at room temperature on both the AV-300 and 500 have a limited operating temperature range of +/- 50°C (323 K – 223 K). Outside of this range, you must use the white ceramic spinner.
- The maximum shim system temperature is +80°C.
- The safe temperature range for the magnet flange / bore is 0 to +50°C.

SECTION B: THE HARDWARE

Overview

The temperature control system works by introducing cooling gas into the probe via a ball-and-socket joint. A heater just inside the probe heats the cooling gas to the desired temperature programmed into a temperature controller. The heated gas then flows upwards through the probe and bathes the NMR sample. A thermocouple situated ~ 1 mm below the sample detects the temperature and reports it to the controller. The temperature controller compares this reported temperature to the target temperature and adjusts the heater current accordingly to maintain the desired temperature.

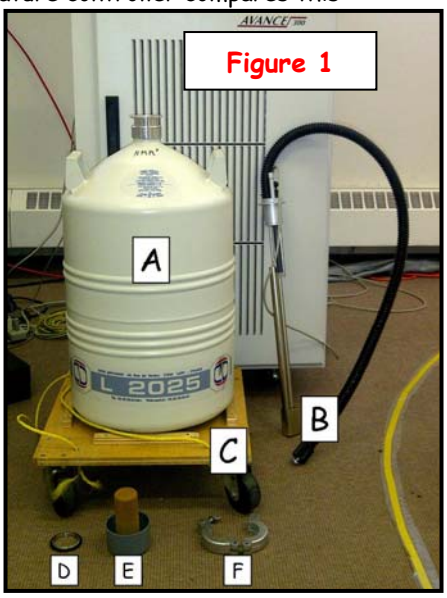


Figure 1

Generation of Nitrogen Cooling Gas

For low temperature experiments, the cooling gas is N_2 which is generated using the equipment shown in Figure 1. A cold stream of nitrogen gas is generated via a liquid nitrogen heater **[B]** immersed in a 25 L dewar **[A]** containing liquid N_2. The amount of flow is determined by the amount of power supplied to the heater and this, in turn, determines the lowest temperature achievable; higher flow → lower temperatures. The cold nitrogen gas flows to the sample through a flexible and insulated transfer arm **[B]** which is connected to the probe at the ball-and-socket joint via a clamp. In order to realize efficient temperature reduction, this connection must be well sealed in order to minimize cooling gas loss to the atmosphere.

Liquid N_2 Level Monitoring

It is important to have sufficient liquid N_2 before starting your low temperature NMR experiments and to have a means of monitoring the liquid level throughout. How long the liquid N_2 lasts depends on several factors including the applied cooling power, the dewar vacuum, and the seal at the connection between the nitrogen transfer arm and probe. Bruker states that as a guide, 25 L of liquid N_2 will last 8 hours at 50% cooling power.

To monitor the liquid level, the nitrogen heater has two integrated level sensors. When the level in the dewar drops below the first sensor, the operator receives a flashing "REFILL" warning. The equipment continues to be fully functional but it is time to either complete your experiments or replenish the liquid N_2 supply. If liquid N_2 is not added and the level drops below the second sensor, the operator now receives the more serious "EMPTY" warning. When this happens, the N_2 heater is disabled as otherwise it would overheat. If additional experiments are required, the operator has no choice but to replenish the liquid nitrogen supply via the refill port.

SECTION C: THE SOFTWARE

A software program called "edte" is available to interface with the temperature controller. The main display tab of the edte program is shown in Figure 2. This is the area you will use to control the sample temperature as well as the nitrogen and probe heaters. A brief description of each area in this display follows:

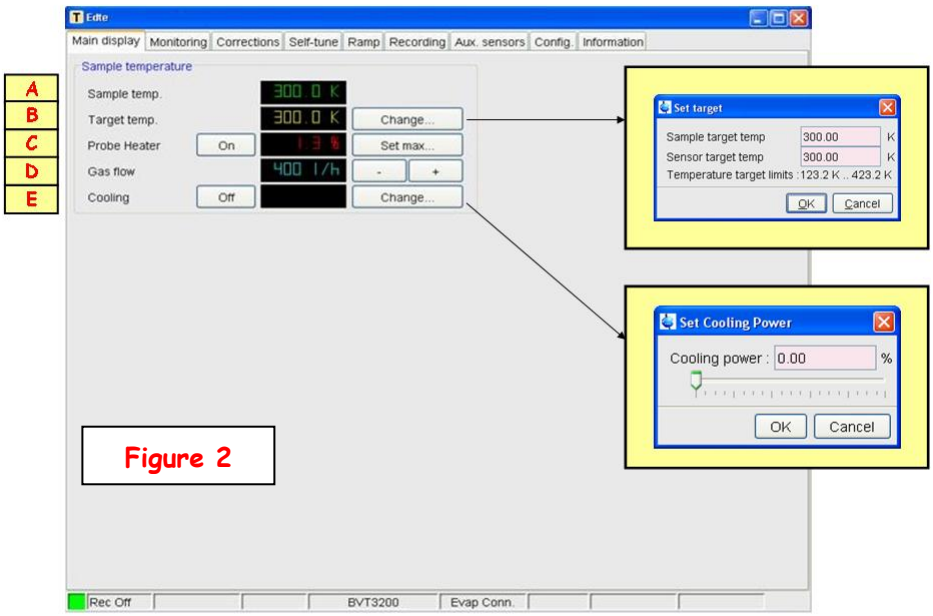


Figure 2

- **[A] Sample temp. Section:** this is the temperature of the sample in Kelvin as reported by the thermocouple.
- **[B] Target temp. Section:** the requested sample temperature. The operator can change the requested temperature by clicking on the Change… button and typing in a new value.
- **[C] Probe Heater Section:** (1) status of the probe heater (ON or OFF). Clicking this button toggles the heater on and off (2) display window which shows "—OFF--"if the heater is disabled or shows the heating power being applied (% maximum power) if the heater is on (3) a button called "Set max…" limits the available heating power to some number below 100%.
- **[D] Gas flow Section:** flow rate in units of L/h. Adjusted using the "+" or "-" buttons beside the flow rate display.
- **[E] Cooling Section:** Shows (1) status of the N_2 heater (ON or OFF). Clicking toggles the N_2 heater on and off (2) the display window either shows "—OFF--" if the N_2 heater is turned off or it shows the power being applied (% maximum power) if the heater is on. A flashing "REFILL" or "EMPTY" warning is shown here if the liquid N_2 level in the dewar gets sufficiently low (3) a button called "Change…" which controls the nitrogen heater power and consequently the cooling capacity. Larger numbers are required here for lower temperatures.

SECTION D: Operating Procedures

Operating Procedures: Lowering the Temperature

Note that you should perform steps (1), (2) and (3) below at least 1.5 hours before your booking on the spectrometer. If you neglect to do this, the N_2 gas flow into the probe may be difficult to control until thermal equilibrium is established. The letter designations in the instructions below refer to Figure 1 (unless otherwise indicated)

(1) Add an appropriate amount of LN_2 to the 25 L dewar **[A]**. Note that the NMR facility has a cart with a pull-string **[C]** which you can use to transport the dewar as it is heavy. **Caution!! Do not bring the cart inside the 5 Gauss stray field line**. Make sure you cap the dewar after adding liquid N_2 with the grey styrofoam cover **[E]**.

(2) Place the o-ring **[D]** on top of the neck of the dewar **[A]** and then slowly immerse the evaporator **[B]** into the dewar as shown in the picture on the right. **Caution!! Liquid N_2 will boil and possibly liquid will spill onto the floor at this point so wear protective clothing and glasses**.

(3) Clamp the evaporator using the metal compression fitting **[F]**.

(4) Remove the autosampler carousel by turning power off to the unit (press the blue power button for 5 seconds) and removing the carousel as demonstrated during

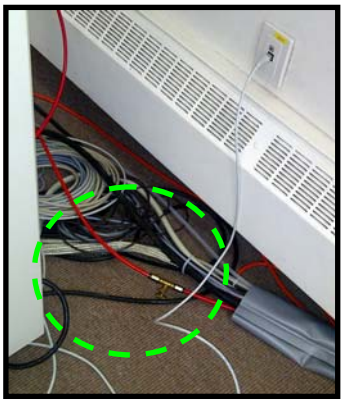
your training session. If you need a reminder, consult the guide in the binder entitled "Installing and Removing AVANCE 300 Autosampler".

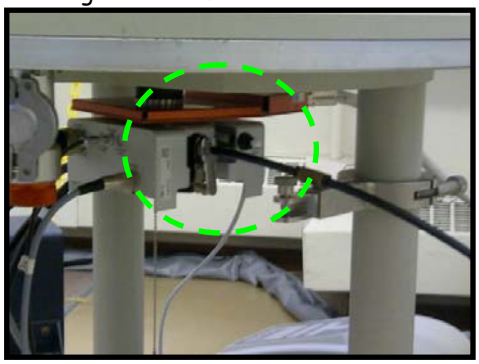
(5) Open the edte software by simply typing "edte" in the Topspin command line.

(6) Turn the probe heater off (if it is on).

(7) Remove the yellow plastic chain from in front of the AV-300 magnet by taking it off one of the wall hooks.

(8) Lower the magnet anti-vibration platform by closing the needle valve located on the floor in back of the console (shown in the figure on the right). The valve is closed by rotating clockwise.





(9) Remove the black air line from the probe (shown in the figure on the left) by loosening and then squeezing the clamp to remove it. **Caution!! Be careful where you place the clamp as it is slightly ferromagnetic.**

(10) Connect the insulated transfer arm from the nitrogen heater to the probe using the clamp on the magnet leg to support it. Position the dewar such that the line into the probe is as straight as possible.

(11) Wrap cotton wool around the probe connection to minimize gas leakage and frost.

(12) Using the ceramic spinner, insert your sample into the magnet (press the "LIFT ON/OFF" button on the BSMS keypad for eject air) and then place the black plastic cap over the bore of the magnet.

(13) Lock and shim as normal and acquire a room temperature spectrum to ensure your sample is what you expect.

(14) In the edte "Target temp" section, click on the Change… button and enter the lowest temperature you plan on studying (remember to stay 10 degrees above your solvent's freezing point).

(15) To start lowering the sample temperature, click the OFF button in the edte Cooling section to turn the N_2 heater ON and then click Change… and apply **35% cooling power**. Experience has shown that 35% will lower the temperature nice and slowly. Remember to stay 10° above your solvent's freezing point!

(16) When the sample temperature is ~5-10° above the target temperature, turn on the probe heater and adjust the cooling power according to the values in Table 1.

(17) When the desired temperature has been reached, you may wish to perform a procedure called **"self-tune"** which is described at the end of this document. This procedure "tunes" the temperature controller and so should result in improved temperature stability/regulation

Table 1	
Target Temperature Range (Kelvin)	**Suggested Cooling Power (%)**
Ambient – 280	5
280 – 270	10
270 – 260	15
260 – 250	20
250 – 240	25
240 – 230	30
230 – 220	35
220 – 210	40
210 – 200	45
200 - 193	50
The numbers in this table are guidelines only!! The actual values will depend on the quality of the probe connection, dewar vacuum quality, etc.	

(18) Wait ~10 minutes for your sample to reach thermal equilibrium and then re-shim and re-tune the probe before running your NMR experiments. Both shimming and probe tuning can change dramatically as a function of temperature.

(19) Once finished at this initial, lowest temperature, increase the set temperature by no more than 15° at a time and wait a few minutes at each step until you are at your next desired temperature. Once the next desired temperature has been reached, continue the sequence of performing a "self-tune" (if desired), wait ~10 minutes for the sample to equilibrate, and then re-shim and re-tune the probe.

Operating Procedures: Returning to Room Temperature after Finishing Low Temperature VT

(1) When your NMR experiments are complete, it is time to return to the normal operating temperature slowly. To begin, increase the temperature to the next highest multiple of 10° and adjust the cooling power according to Table 1. Wait for a few minutes and then continue "upwards" in multiples of 10°, waiting a few minutes at each stop.

Caution!! Do not eject your sample yet; wait until the probe and sample are ~ 290 K. Note that when returning to room temperature, some have asked why not turn the cooling power immediately to 0%; this is not advised as it is more difficult to control the return to room temperature in a predictable and controlled manner.

(2) Continue this process until you have waited for 5 minutes at 290 K. Set the cooling power to 0% and turn the N_2 heater off.

(3) Turn off the probe heater and remove the transfer arm from the probe very carefully by removing the wool and clamp and then pulling straight back. **Caution!! Do not use much pulling force.** If it does not pull away easily, wait 5-10 more minutes for additional thawing.

(4) Re-attach the room temperature air line to the probe.

(5) Reinstall the autosampler carousel and restore power to the autosampler.

(6) Raise the anti-vibration magnet platform by opening the valve on the floor behind the console, replace the plastic yellow chain across the front of the magnet (remember that the link to place on the hook is marked), and put the stop-step ladder back in the normal position in front of the magnet.

(7) Turn the probe heater back on.

(8) Remove the black plastic cap from the bore of the magnet (return it to the basket) and eject your sample.

(9) Adjust the temperature to 300 K and increase the air flow to 1070 l/h using the '+' button in the "Gas flow" section of edte.

(10) You should now have at least another 1/2 hour of time signed out in FACES for the system to re-equilibrate thermally. **You and only you are responsible to have the probe at room temperature before the next person uses the spectrometer!**

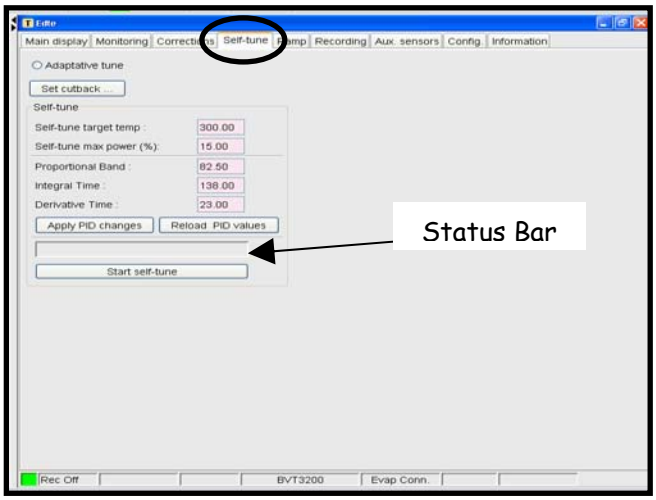
(11) You can determine if equilibrium has been re-established by turning the air flow to 0 l/h and seeing if room temperature is maintained (293 K). If not, increase the air flow back to 1070 l/h and manually turn the probe heater back on (it is automatically disabled when the air flow goes to zero). If it is, increase the air flow to 400 l/h and manually turn the probe heater back on.

(12) If you performed the self-tune procedure, you must read in the standard temperature control parameters for the day-to-day operating temperature of 300 K. In edte, click on the "Config." tab and at the bottom in the "Miscellaneous" section; click the "Load configuration …" button. A list of configuration files should now be present; select the file bbfo_300K_400lh.tcf and click Open to load the controller parameters. Note that if you did not self-tune, this step may be skipped.

(13) Before Finishing: check that the probe heater is on, the gas flow is at 400 l/h, and the temperature is set to 300 K. Ensure all items used for low VT experiments are returned to the basket (2 ceramic spinners in blue plastic case, cotton wool, Styrofoam cap, hair dryer, black). Put the basket back in the AV-300 cabinet on the wall behind the spectrometer.

Performing a "Self-Tune"

Self-tune is a procedure built into the edte software which tunes the temperature controller at a given temperature. In theory, once self-tune is performed, the controller will provide more stable temperature control (limited fluctuations) as well as a quicker response to any deviation from the desired temperature. You should consider performing a self-tune when you change the cooling gas flow rate (i.e. anytime you change the cooling power for low temperature experiments) as well as whenever you change the target temperature by more than 10°. To perform a self-tune, perform the following steps in the edte software:



(1) Click on the "Self-tune" Tab in edte to open up the self-tune interface (shown in the Figure on the right).

(2) The "Self-tune target temp" and "Self-tune max power (%)" are by default set to appropriate values and shouldn't be changed.

(3) Start by clicking on the "Start self-tune" button. Once you do this, a message "Self-tune is running" will be displayed in the status bar and the start button changes to a "Stop self-tune" button which you can click to stop the tune early.

(4) The tune takes on the order of a few minutes to complete. You will know it's finished when the status bar message area is empty and the "Stop self-tune" button changes back to a "Start self-tune" button.