Variable Temperature NMR Experiments

1. Introduction to Variable Temperature (VT) NMR

1.1 What is VT NMR?

NMR experiments in the Department of Chemistry are normally run at an ambient temperature of 25°C (298K). It is sometimes necessary to run experiments at temperatures significantly higher or lower than ambient. Such variable temperature (VT) experiments are used for a number of reasons, mainly to provide insights into the dynamic and kinetic behaviour of molecules, or to simplify spectra for compounds that are undergoing conformational exchange e.g. rotamers.

Changing the probe temperature away from ambient has the potential to cause serious damage to an NMR spectrometer. Therefore please be careful when performing a VT NMR experiment, and take the time to familiarise yourself with the contents of this document.

You must NOT perform any VT NMR experiments unless you have been trained to do so.

Common reasons for running high temperature NMR:

- Resolving (sharpening) broad peaks due to exchange processes
- Following chemical or conformational exchange processes
- Following a high temperature reaction
- Solubility issues

Common reasons for running low temperature NMR:

- Following chemical or conformational exchange processes
- Trapping reaction intermediates
- Following a low temperature reaction
- Chemical instability

1.2 Important considerations for VT NMR

Please follow these instructions carefully. If you are unsure of anything ***ask a member of the NMR staff for assistance.***

1. **Available instruments:** The instruments that are available for VT NMR work are the AVB400, AVB500, AVX500 and AVD500, all of which are located in the NMR suite in the CRL basement, or Venus400 on the 2nd floor. The AVB500 and AVX500 instruments have chiller (fridge) attachments for (limited) sample cooling.

2. **Temperature limits:** We generally advise operating temperatures of no higher than 100°C (373K) and no lower than -80°C (193K).

3. **Appropriate tubes:** It is essential that you use only Class A glass tubes (“pyrex”) for VT work, such as Wilmad 507 or above. Cheaper (Class B) glass will deform at temperature extremes and may fracture; this includes the commonly used “disposal” NMR tubes which are not suitable for VT work.

4. **Appropriate spinners:** When performing VT NMR you must always use the brown PEEK or white ceramic spinner (turbine) (Figure 1) with either high or low temperatures. This is because the normal turbines can be damaged at extremes of temperature.

Note: Please be very careful when inserting an NMR tube into the ceramic spinners, as there is often a very tight fit and a consequent risk of injury due to breakage of the tube. The best way to insert an NMR tube into a ceramic spinner is by **gently** and **slowly** applying a twisting pressure to the tube during insertion.

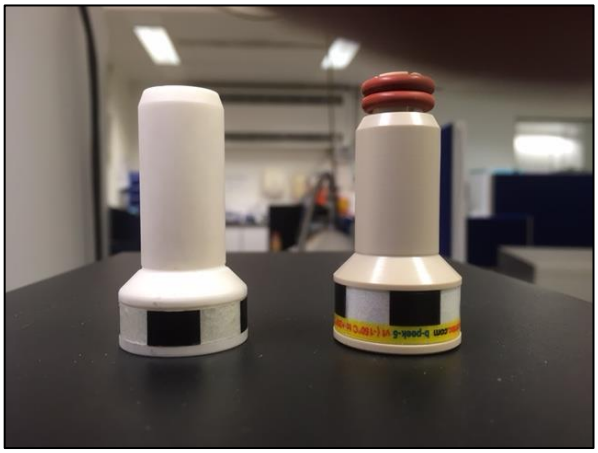


Figure 1: Appropriate spinners for VT NMR (*Left*: ceramic, *Right*: PEEK).

5. **Appropriate solvent:** Make sure that you use a solvent with an appropriate boiling or freezing point - i.e. it will not boil or freeze at your target temperature. Although an obvious statement, numerous chemists have been known to make this mistake. You must not get any closer than 10^{o}C to the boiling or freezing point (please see the NMR solvent sheets near the instruments for these). In addition, it is good practice not to use a sealed NMR tube when performing high temperature NMR experiments. The most common solvents for high-temperature NMR are DMSO (BP 189 ^{o}C) or Toluene (BP 111 ^{o}C), and for low-temperature are CD_2Cl_2 (MP -95 ^{o}C), MeOD (MP -98 ^{o}C) or Toluene (MP -95 ^{o}C).

6. **Allow sufficient time:** When planning VT experiments, please make sure you have enough instrument time to allow the system to equilibrate at ambient temperature before the next user; this will usually require an additional 20-30 minutes after your experiments.

7. **Sample Solubility:** Bear in mind that solubility can be reduced at low temperatures. Therefore if you suspect that your sample is coming out of solution at low temperature, it may be necessary to either dilute your sample or to use a higher temperature.

8. If you want to start measurement immediately at a certain temperature (eg for reaction monitoring), you should have a "blank" NMR tube, with an equivalent volume of your solvent. When you have reached the desired temperature, tune, match and shim on this blank sample. You can then exchange this with your sample, which you should have cooled/heated to the correct or approximate temperature externally. You can then either start your experiment immediately without further shimming, or you can use a "quick" shimming routine, by typing in "topshim convcomp ordmax=3" instead of the usual "tshim" or "tshimvt" command.

1.3 General procedures

It is usual procedure to run VT experiments under manual operation only using manual sample handling and **not** through ICONNMR.

1.3.1 Removing or disabling the autosampler

To stop and disconnect the autosampler device on the AVB400 and AVB500, hold down the on/off button until the light goes out, then gently **lift** the carousel off the top of the magnet (Figure 2). Make sure that you place it safely on a table. The autosampler on the AVX500/AVD500 or venus400 cannot be removed. Instead, it is sufficient to turn it off by depressing the red on/off button (Figure 3).

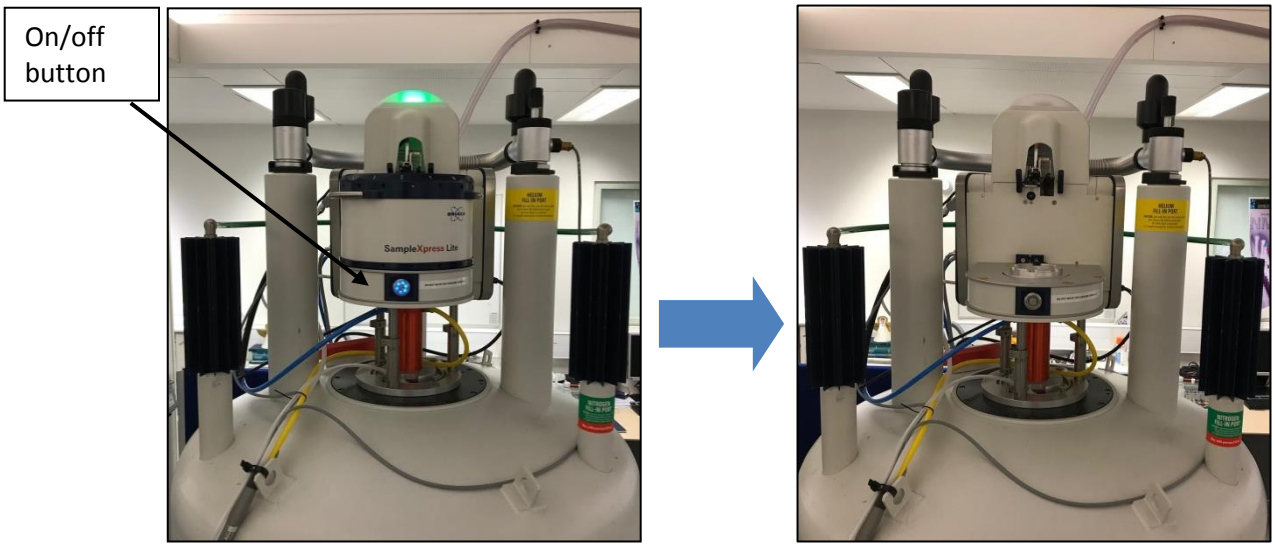


Figure 2: Removing the autosampler carousel from the *SampleXpress Lite*.

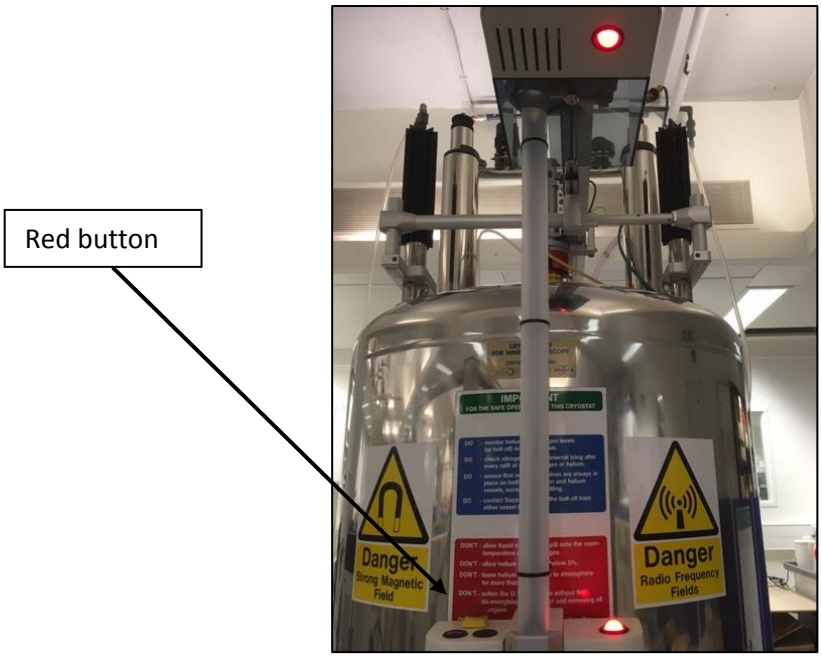


Figure 3: Disabling the *SampleCase* autosampler. The red light indicates the unit is disabled.

1.3.2 Manual sample insertion

To manually insert your sample, open the BSMS display by either typing "bsmsdisp" into the command line in Topspin, or by clicking on the keypad icon  . In the "Sample" section under the "Main" tab, click on the "Lift" button, which should turn green (Figure 4). **Wait until you can hear air coming out of the bore on top of the magnet**. Place your sample into the bore, and click on the "Lift" button once again. The button colour will change to grey, and the sample should slowly descend into the magnet.

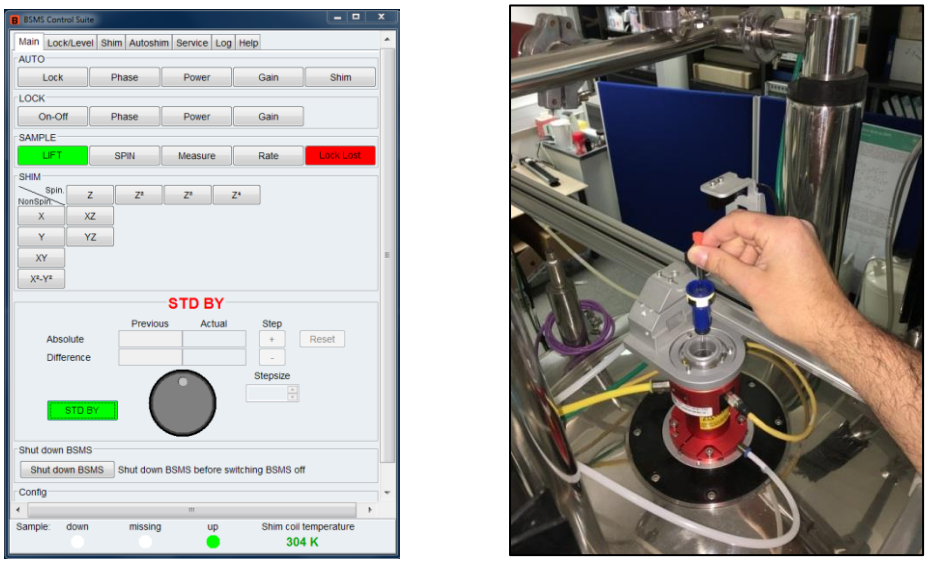


Figure 4: Manual sample insertion: *Left*: the BSMS panel and *Right*: sample insertion to magnet bore.

2. High Temperature Experiments

2.1 Overview

The upper temperature limit for experiments is typically 100°C. In occasional circumstances it may be possible to exceed 100°C for short periods of time, but this must only be done with the agreement of the NMR staff. Once again, you are reminded to check the boiling point of your solvent, and to **not** use a temperature within 10° of this boiling point. The shim coil system has an upper limit of 80°C and this must never be exceeded.

2.2 Preparation

2.2.1 Inserting your sample

You must use **manual** mode to insert your sample, which should now have the appropriate turbine attached (see section 1.3). Remove or disable the autosampler as described in section 1.3.1. Insert your sample into the magnet as described in section 1.3.2.

2.2.2 Run a room temperature experiment.

Now that your sample is in the magnet, it is good practice to run an experiment at ambient temperature, to ensure sample integrity and optimal spectrometer operation.

2.3 Establishing high temperature

2.3.1 Switch off the chiller unit

If using the AVB500 or AVX500, it is important to switch off the chiller unit before any heat is applied to the probe. This is done by selecting the "Flush/0" setting, as shown in Figure 5.

Figure 5: Turning off the chiller units on the AVB500 and AVX500- turn dial to ***Flush.***

2.3.2 Set the correct temperature calibration

To access the VT temperature control unit, either type **edte** or choose **T** on the top right hand corner of TOPSPIN, or double click the sample temperature window on the bottom right-hand side.

Load the temperature correction and calibration settings, according to table 1. The temperature correction settings are accessible from the "*Correction*" tab of the Temperature Control Panel, as shown in Figure 6. Select the calibration file for the probe and desired temperature range, and click on the "Set" button. Make sure there is a tick in the box for "Enable temperature correction with these settings".

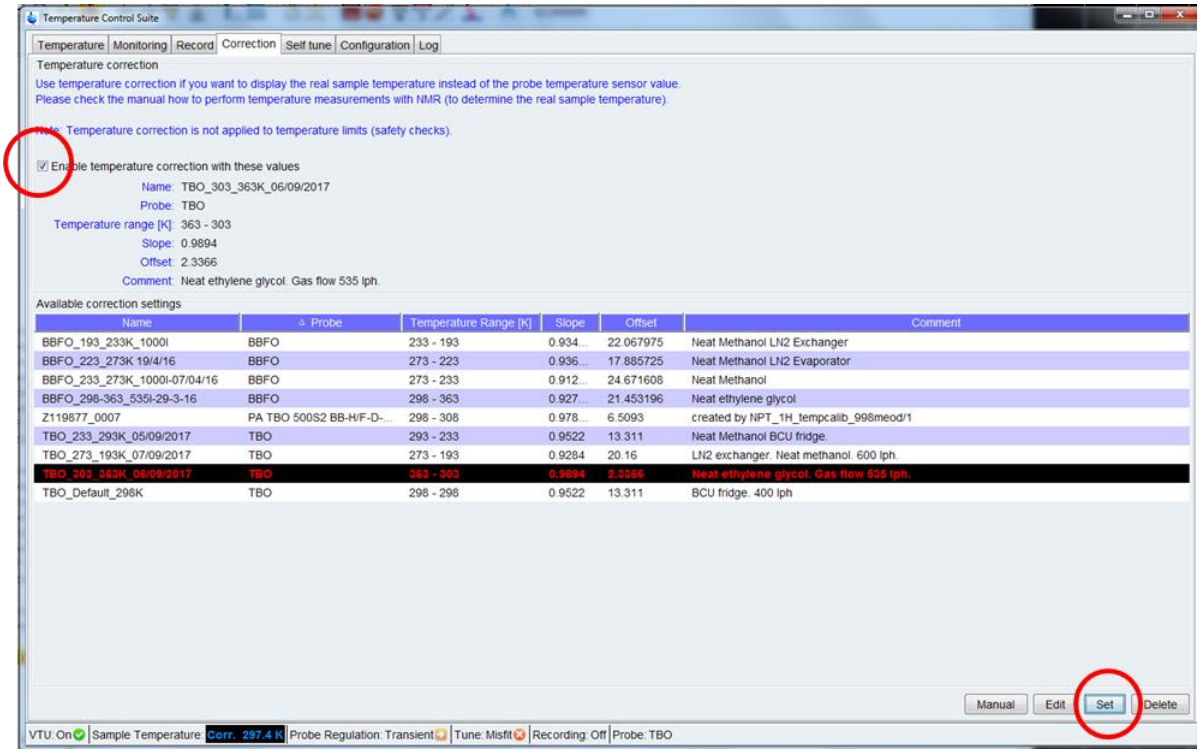


Figure 6: Setting the temperature calibration file.

2.3.3 Setting the temperature and gas flow

The temperature control panel is shown in Figure 7, viewed with the "*Temperature*" tab. The VTU status should be on. Set the Target Gas Flow to a value specified in Table 1. It is **extremely important** to increase this gas flow, as failure to do so could overheat the probe and shims. Set the Target Temperature to the desired value using the **Set** button. It is advisable to change temperature in steps of 10-20^{o}C only to minimise thermal shock to the probe, and progress step-wise to your desired temperature.

Note: the Sample Temperature indicator is colour coded. When the sample temperature is regulated, it turns green. When the temperature is too low or too high, the colour becomes blue or red respectively.

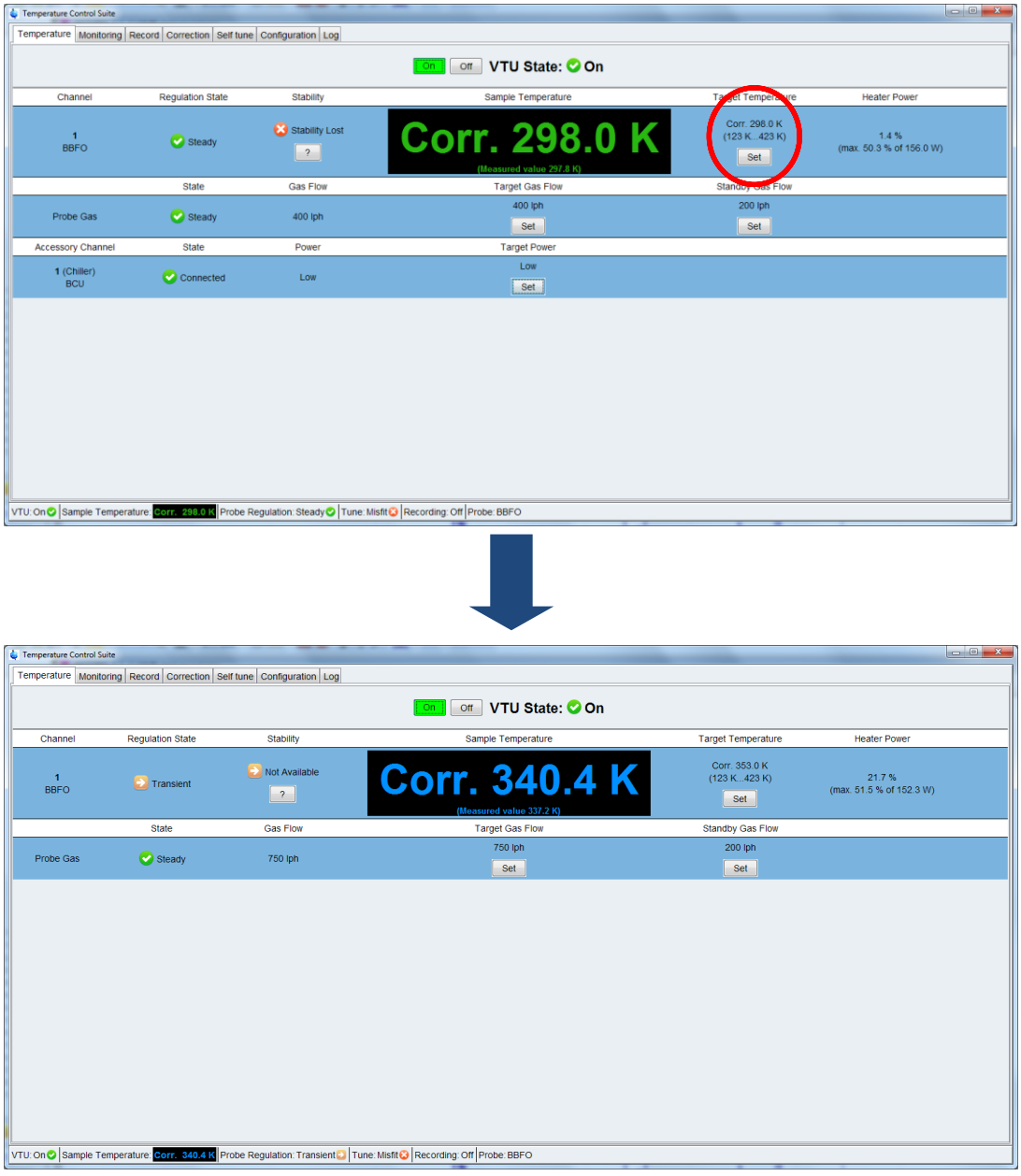


Figure 7: Setting the temperature for high-temperature NMR.

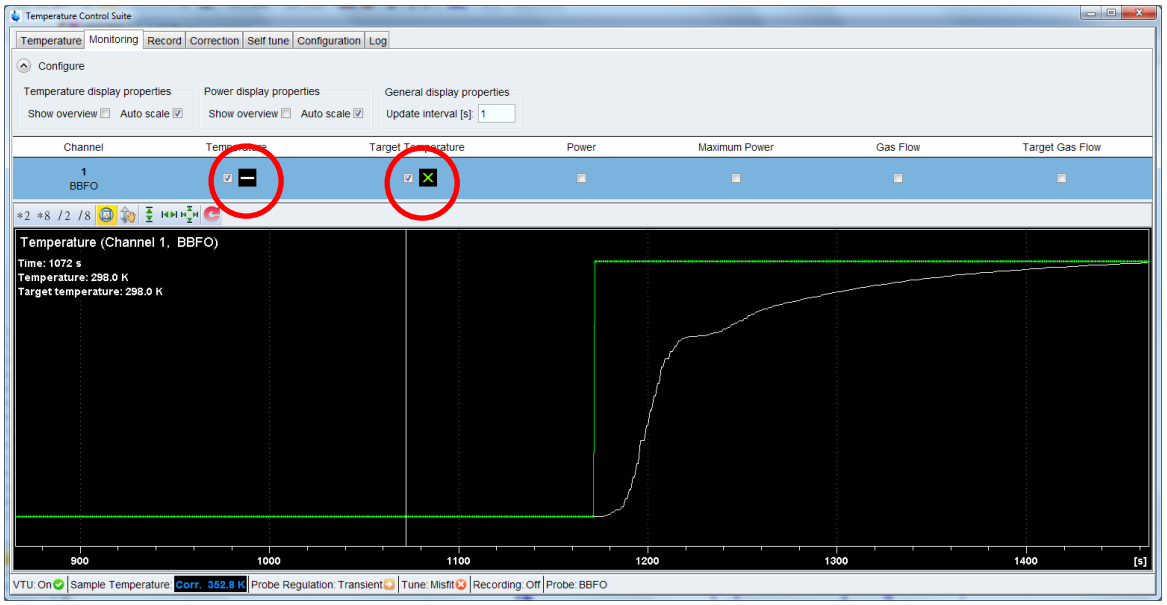
Spectrometer	Gas Flow (lph)	Calibration file
AVB400	535	BBO_298_363_535l
AVB500	535	bbfo_298-368_535l.cor
AVX500	535	TBO_303_363K

Table 1. Gas flow settings and calibration files for high-temperature NMR.

2.3.4 Equilibrating the temperature

Allow the temperature to equilibrate at the final temperature, which can take up to 20 minutes. A stable temperature is indicated by a completely horizontal line in the "*Monitoring*" tab, when the "Temperature" and "Target Temperature" fields are selected (Figure 8A), and by a green tick in the Temperature Control Panel (Figure 8B).

(a)



(b)

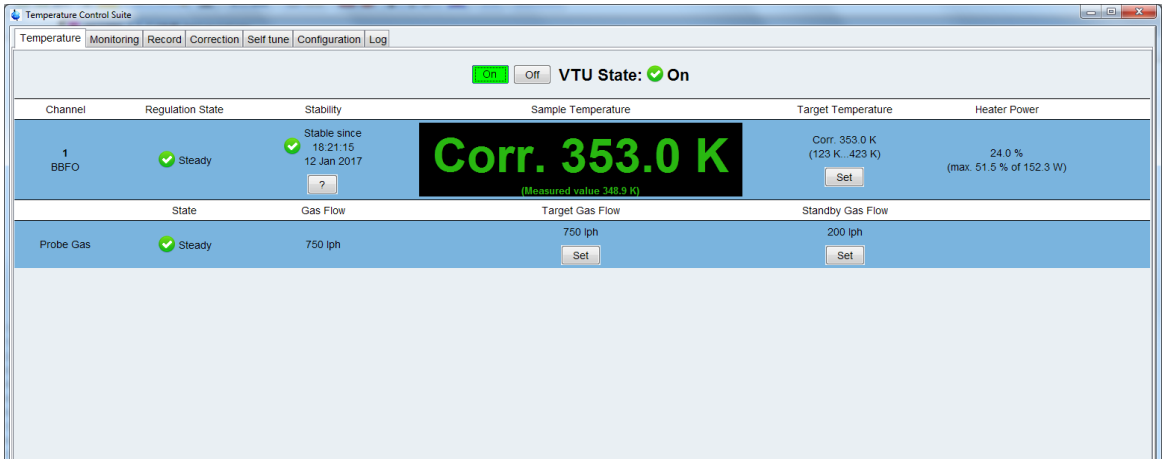


Figure 8: a) Monitoring the temperature during equilibration, b) display when temperature stabilised.

2.3.5 Running high temperature NMR experiments

Now that the temperature has equilibrated, you are ready to perform your high temperature NMR experiment. Data acquisition is done as normal, including all locking, tuning/matching, and shimming steps. For shimming, you should use the ***tshimvt*** routine in place of ***tshim***. *If leaving the instrument unattended, it is advisable to leave a note warning the instrument is running in VT mode*.

2.3.6 Returning the spectrometer to ambient temperature

Having performed all your high temperature experiments, **please remember** to return the VT unit back to ambient temperature by carrying out the above procedures in reverse:

a) Decrease the temperature using set button to 298K.
b) Decrease the airflow back to 400lph.
c) Set the chiller/fridge unit back to the original "1" position.
d) Set the temperature correction back to "default_298K"
e) Wait until the temperature is near ambient.
f) Eject the sample manually from probe when it is safe to do so.
g) Replace the autosampler to its original state in the 'on' position for the next user.

3. Low Temperature Experiments using the Chiller

3.1 Overview

There are two ways to perform a low temperature experiment. For temperatures from room temperature down to -40°C (233K) on AVX500, it is advisable to use the attached chiller unit (BCU II) as described here. For temperatures below -40°C (233K), and for all low temperature experiments on all other instruments, it is necessary to use a liquid nitrogen exchanger (see section 4).

3.2 Preparation

3.2.1 Inserting your sample

You must use **manual** mode to insert your sample, which should now have the appropriate turbine attached (see section 1.2). Remove the autosampler if using the AVB400 / AVB500, or disable the autosampler if using the AVX500, AVD500 or venus400, as described in section 1.3.1. Insert your sample into the magnet as described in section 1.3.2.

3.2.2 Run a room temperature experiment

Now that your sample is in the magnet, it is good practice to run an experiment at ambient temperature, to ensure sample integrity and optimal spectrometer operation.

3.3 Low temperature experiments using the chiller unit on the AVX500.

3.3.1 Overview

The BCU II chiller unit may be used for temperatures down to -40°C. In general, the use of the chiller unit is less complicated and time-consuming than the use of a nitrogen exchanger. Therefore for temperatures down to -40°C we advise using this procedure. A low-temperature chiller unit is only available on the AVX500 (that on the AVB500 cools to only ~5^{o}C). If you are using other instruments for low-temperature NMR, you should use a nitrogen exchanger as described in section 4.

3.3.2 Adjust the chiller unit

Switch the chiller unit to the "2" setting, as shown in Figure 9.

Figure 9: Switch the chiller unit to the "2" position.

3.3.3 Setting the temperature and gas flow

Open the VT temperature control window. Set the appropriate temperature calibration file, as specified in Table 2. Immediately after this, set the Target Gas Flow to a value specified in Table 3.
Set the Target Temperature to the desired value using the **Set** button. It is advisable to change temperature in steps of 10-20^{o}C only to minimise thermal shock to the probe, and progress step-wise to your desired temperature.

Spectrometer	Calibration file
AVX500	TBO_233_293K

Table 2. Calibration files for low-temperature NMR using the chiller unit.

Spectrometer	Temperature (K)	Gas Flow (lph)
AVX500	293	400
	283	500
	273	600
	263	700
	253	800
	243	800
	233	1000

Table 3. Gas flow settings for low-temperature NMR using the chiller unit.

3.3.4 Temperature equilibration

Allow the temperature to equilibrate, which can take up to 20 minutes and can be checked using the "*Monitoring*" tab. Temperature stability is achieved when a completely horizontal line is achieved in the Temperature Monitoring Panel and when a green tick appears in the Temperature Control Panel (Figure 8 above).

Note: the Sample Temperature indicator is colour coded. When the sample temperature is regulated, it turns green. When the temperature is too low or too high, the colour becomes blue or red respectively.

3.3.5 Running low temperature NMR experiments

Now that the temperature has equilibrated, you are ready to perform your low temperature NMR experiment. Data acquisition is done as normal, including all locking, tuning/matching, and shimming steps. For shimming, you should use the ***tshimvt*** routine in place of ***tshim***. *If leaving the instrument unattended, it is advisable to leave a note warning the instrument is running in VT mode*.

3.3.6 Returning the spectrometer to ambient temperature

Having performed all your low temperature experiments, **please remember** to return the VT unit back to ambient temperature by carrying out the above procedures in reverse:

a) Set the Chiller/fridge unit back to the original "1" position.
b) Increase the temperature using set button back toward 298K, in steps of 10°.
c) Decrease the airflow back to 400 lph, in steps according to table 3.
d) Set the temperature correction back to "default_298K"
e) Wait until the temperature is near ambient.
f) Eject the sample manually from probe when it is safe to do so.
g) Replace the autosampler to its original state in the 'on' position for the next user.

4. Low Temperature Experiments using the Nitrogen Exchanger

4.1 Overview

For temperatures below -40°C (down to -80°C) on the AVX500, and for all low temperature work down to -80°C on other instruments, it is necessary to use a nitrogen exchanger. This device uses a tank of liquid nitrogen to cool a flow of nitrogen gas, which is then fed to the probe.

4.2 Liquid Nitrogen Dewar

The first step is to fill a 25-litre LN_2 dewar (Figure 10) with liquid nitrogen, using a filling station, ensuring to follow the safety procedures for handling liquid cryogens. The closest filling station to the NMR laboratory is located adjacent to the goods lift. Use the wooden trolley shown in Figure 10 to transport the dewar to and from the filling station. Once full, return the dewar to the NMR laboratory, remove it from the trolley, and place it adjacent to the magnet, as shown in Figure 10.

Note: Follow all appropriate safety guidelines, including the wearing of safety glasses and thermal gloves, when filling the dewar with liquid nitrogen. If you are transporting the dewar to a filling station on another floor, **NEVER** travel with the dewar in a lift.

Figure 10: *Left*: Liquid nitrogen dewar and trolley. *Right*: Appropriate positioning of liquid nitrogen dewar with respect to the magnet.

4.3 Low temperature experiments

4.3.1 Inserting your sample

You must use **manual** mode to insert your sample in an **appropriate tube**, which should now have the **appropriate turbine** attached (see Section 1.2). Remove or disable the autosampler as described in Section 1.3.1. Insert your sample into the magnet as described in Section 1.3.2.

4.3.2 Run a room temperature experiment

Now that your sample is in the magnet, it is good practice to run an experiment at ambient temperature, to ensure sample integrity and optimal spectrometer operation. Remember to use a correct VT spinner, as only these are tolerant of temperature extremes.

4.3.3 Prepare the transfer Line

Before and after use, the transfer line must be flushed through with dry gas in order to remove any condensation (see Figures 11 and 12). Use the nitrogen gas line adjacent to the AVB400. First, place the nitrogen exchanger in an empty dewar. Then connect the ball joint on the nitrogen gas cable securely to the gas inlet valve on the LN_2 exchanger, and tighten the screw on the clamp to ensure a good seal. Open the N_2 gas valve ***very slightly and carefully***, so that you can hear N_2 gas flowing out of the N_2 exchanger. Leave the line flushing for 5 minutes.

Figure 11: Nitrogen exchanger and transfer line for low temperature NMR.

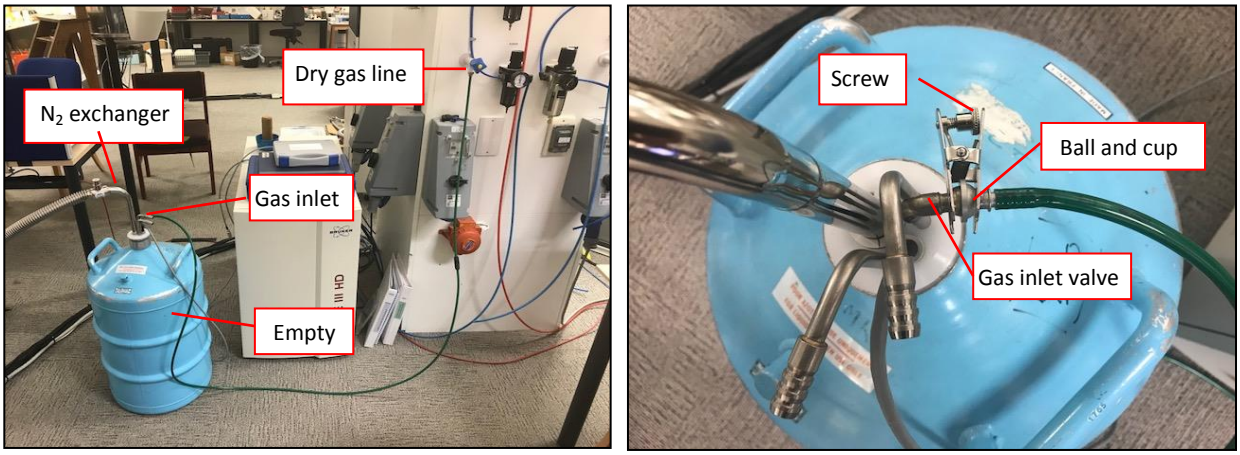


Figure 12: Flush dry gas through the transfer line.

4.3.4 Connect the N_2 transfer line

If you are using an instrument equipped with a chiller unit (BCU) you should set this to "flush" mode and leave this for 5 mins to purge the BCU. You will then need to detach the N_2 gas feed into the chiller and connect this to the gas line adapter ("cup") that connects to the N_2 exchanger (Figure 12 above). If the instrument does not use a chiller, you will only need to connect the usual N_2 gas line to the "cup" adapter. Then, to set-up the N_2 transfer line, follow these steps:

1. In TopSpin open the Temperature Control Suite by typing "edte" in the command line, or by clicking on the  icon. Turn off the Variable Temperature Unit (VTU) as shown in Figure 13.

Note: it is important to turn off the VTU before connecting the LN_2 transfer line in order to prevent overheating of the probe.

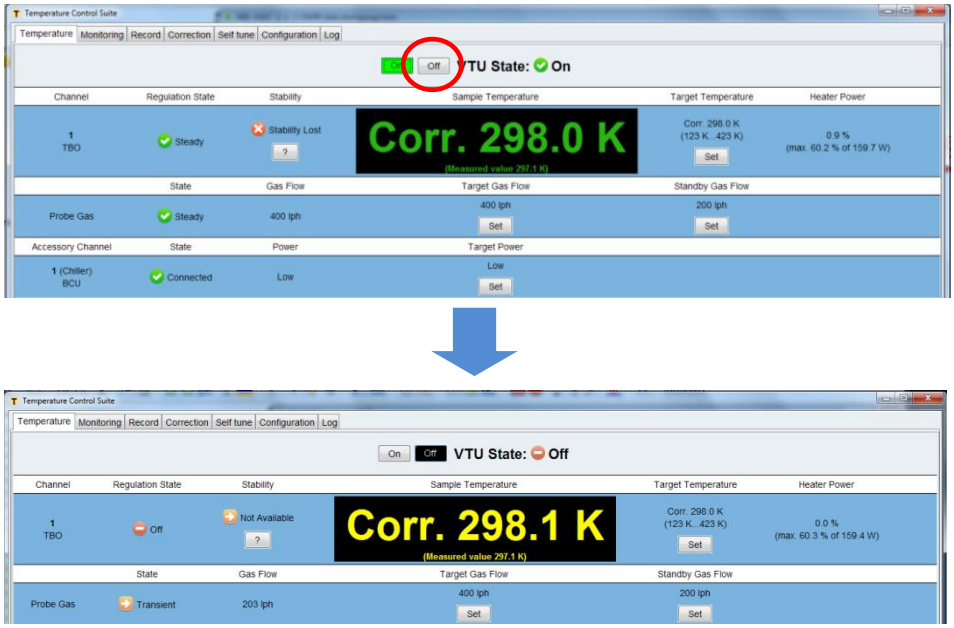


Figure 13: Turning off the VTU.

2. Disconnect either the black VT gas connector on BCU hose from the probe (Figure 14a) or disconnect and remove the VT adaptor (Figure 14b), according to instrument set-up. Connect the gas line terminated with the cup adaptor to the probe N_2 gas line (Figure 14b, bottom right).

(a) (b)

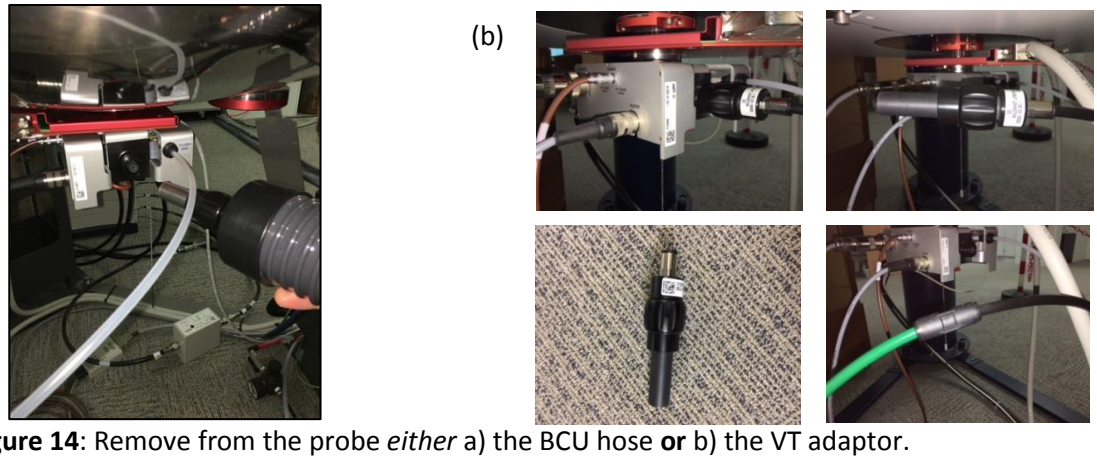


Figure 14: Remove from the probe *either* a) the BCU hose **or** b) the VT adaptor.

3. SLOWLY insert the coils of the nitrogen gas exchanger into the LN_2 dewar, as shown in Figure 15.

Note: Make sure the two nozzles on the cap are pointing away from you, as these can spray jets of liquid nitrogen if the probe is inserted too quickly. Make sure that you wear **gloves** and **safety glasses** while doing this.

Figure 15: Inserting the N_2 exchanger into the LN_2 Dewar.

4. Attach the transfer line to the probe **GENTLY**. Keep the transfer line horizontal when inserting it into the probe. There should be no resistance if the positioning of the dewar and transfer line is correct (see Figure 16). Once the transfer line is in place, screw the end into place.

Note: never exert force to push the transfer line into the probe. Ask for help if needed.

Figure 16: Insertion of the N_2 exchanger transfer line into the probe.

5. Connect the N_2 gas supply line to the N_2 exchanger unit using a “cup” adapter and clamp this in place (see Figure 12 above).

4.3.5 Setup for low temperature NMR

To set up your low temperature NMR experiment(s), follow these steps:

1. In the Temperature Control Suite, click on the "*Correction*" tab. Select the appropriate temperature correction file name, as specified in Table 4, and click on the **Set** button (Figure 17).

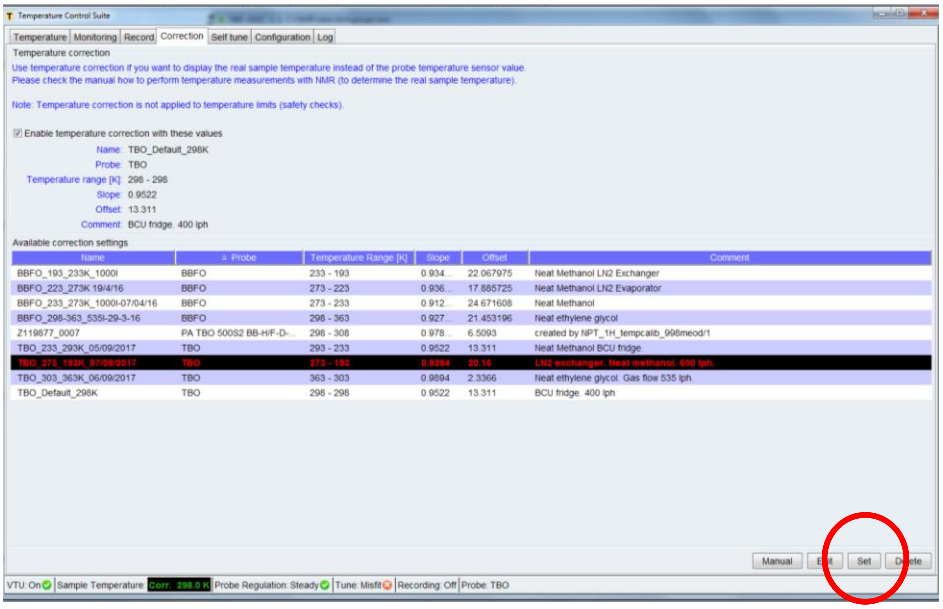


Figure 17: Selection of temperature correction parameters.

2. In the "*Temperature*" tab of the Temperature Control Suite, set the appropriate gas flow rate according to Table 4 then the desired temperature by clicking on the **Set** button, entering the temperature, and clicking on the **OK** button. Remember to decrease temperature in progressive steps of ~ 20°C.

Spectrometer	Gas Flow (lph)	Calibration file
AVB400	1000	BBO_193_273_1000l
AVB500	600	BBFO_233-293 535 LPH.cor
AVX500	600	TBO_193_273K

Table 4. Temperature calibration files and gas flow settings for low temperature NMR.

3. Turn on the VTU by clicking the **ON** button in the top row of the "*Temperature*" tab (Figure 18). Allow the temperature to decrease to the set point and then reduce this further in stages as required.

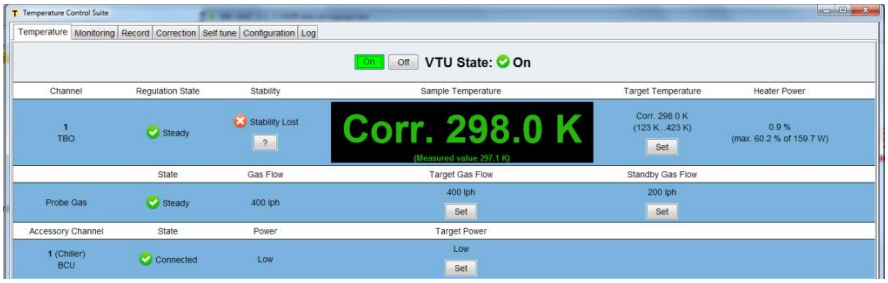


Figure 18: Turning on the VTU

4. Allow the temperature to equilibrate, which can take up to 20 minutes and can be checked using the "*Monitoring*" tab. Temperature stability is achieved when a completely horizontal line is achieved in the Temperature Monitoring Panel and when a green tick appears in the Temperature Control Panel (Figure 8 above).

Note: the Sample Temperature indicator is colour coded. When the sample temperature is regulated, it turns green. When the temperature is too low or too high, the colour becomes blue or red respectively.

Now that the temperature has equilibrated, you are ready to perform your low temperature NMR experiment. Data acquisition is done as normal, including all locking, tuning/matching, and shimming steps. For shimming, you should use the ***tshimvt*** routine in place of ***tshim***. *If leaving the instrument unattended, it is advisable to leave a note warning the instrument is running in VT mode*.

4.3.6 Returning the system to room temperature

Having performed all your low temperature experiments, **please remember** to return the VT unit back to ambient temperature by carrying out the above procedures in reverse:

a) Set the temperature correction back to "default_298K"
b) Increase the temperature using **set** button to 298K, in steps of ~10°.
c) Decrease the airflow back to 400 lph.
d) When the temperature reaches 298K, turn the VTU off by clicking the **Off** button in the **Temperature** tab of the Temperature Control Suite.
e) Disconnect the nitrogen transfer line from the probe and detached the N_2 gas line.
f) Connect the N_2 gas line to the black VT adaptor and attach this to the probe, making sure to do this as quickly and safely possible [NB: If a chiller is available, reconnect the N_2 gas feed to the chiller and set the unit back to the original "1" position].
g) Turn the VTU on by clicking the **On** button in the **Temperature** tab of the Temperature Control Suite.
h) Eject the sample manually from when it is safe to do so.
i) Replace the autosampler to its original state in the 'on' position for the next user.

4.3.7 Flush the N_2 exchanger line

After using the N_2 exchanger it is advisable to flush the line with dry gas to prevent the build-up of condensation inside the line, which could lead to ice formation and blockage on subsequent low-temperature use. Connect the exchanger as in Figure 12 above and follow the procedure described in section 4.3.3 to purge the line.

If in doubt about any aspect of VT NMR, please consult a member of the NMR staff for advice or assistance.

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